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THE ANALOGY BETWEEN FACTORIAL EXPERIMENTATION AND BALANCED MULTI-POINT LINKAGE TESTS

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1. INTRODUCTION

IT was pointed out by Fisher (1952) in the Bateson lecture that the factorial method of experimentation, now used extensively in agriculture and other fields of research, derives its name and structure from the simultaneous segregation of Mendelian characters.

Kempthorne (1955) stressed the genetic origins of the ideas of factorial experimentation when he used these techniques in the splitting up of the genetic variance into components in a system involving the average effects and interactions of unspecified polygenes. In this paper, however, we shall consider major gene effects and their interactions such as are available from a multiple linkage experiment.

In recent years multiple linkage data have been analysed with series of simple χ^2 tests. A discussion of these has been given by Fisher (1949), Parsons (1957, 1958) and Wallace (1947, 1957). In this paper, by applying the principles of factorial analysis, we are attempting to give an alternative and rather more integrated method of analysis.

2. THE FACTORIAL SCHEME

In the data obtained from a backcross linkage test involving the three factors *A*, *B*, *C* and their recessive alleles, each factor will be either heterozygous *Aa*, *Bb*, *Cc* or homozygous *aa*, *bb*, *cc*. This is directly analogous to three treatments at two levels in a 2^3 factorial experiment in which there are eight possible treatments which can be represented as 1, *a*, *b*, *c*, *ab*, *ac*, *bc*, *abc*. The letters *a*, *b*, *c* denote one of the treatment levels and may be taken to represent the recessive homozygotes *aa*, *bb*, *cc* in the genetical analogy. Thus the treatment effects of the factorial experiment correspond to the genotypic viabilities. The main effect in the factorial experiment corresponds to the main viability effect of a gene and interactions between main effects to viability interactions.

The blocks in the factorial experiment correspond to the modes of gamete formation. If each genotype is represented equally in each mode of gamete formation (or block), we can carry out an analysis of variance in the usual way, and hence obtain an analysis of χ^2, giving measures of the viability effects and interactions, and of the recombination effect (block effect). This implies that all the possible multiple heterozygotes must be used as parents, and that each must

contribute equally to the data. In this case the experiment may be called " orthogonally balanced ". A sum of squares (S.S.), and hence a χ^2 for the differences in parental heterozygote contributions can be calculated which is a measure of the departure from orthogonality. Yates and Cochran (1938) have shown in similar situations that such a S.S. may be adequate to account for the non-orthogonality provided that deviations from orthogonality are not too severe. If there are severe deviations, a weighted analysis ought to be used. In sections 3 and 4 a full analysis of χ^2 for data from three-point backcross experiments will be described.

As in agricultural factorial experiments, the higher-order viability interactions may, intuitively, be expected to be of no importance. Even if information were lacking on some of the higher-order interactions it is, nevertheless, probable that the estimates of the recombination fractions would not in fact be biased. In agricultural factorial experiments information on higher-order interactions is often sacrificed so as to be able to use smaller blocks, this process being termed confounding (Fisher, 1935-53), and the same may be done in a genetical linkage experiment.

For example, in a four-point test, the eight possible quadruple heterozygotes can be divided into two sets of four by confounding the *abcd* or 4-factor interaction where *d* represents the fourth factor. This interaction can be found by evaluating $(a-1)\ (b-1)\ (c-1)\ (d-1)$ which equals:

$$1-a-b-c-d+ab+ac+ad+bc+bd+cd-abc-abd-acd-bcd+abcd$$

Confounding this interaction means that the terms of this expansion with negative signs (1 and 3 factor effects) are in one block, and those with positive (0, 2, and 4 factor effects) in the other. Considering those with negative sign, it can be seen that *a*, *b*, *c*, *d* represent the same quadruple heterozygotes as *bcd*, *acd*, *abd*, *abc* so that one set would be:—

$$\frac{aBCD}{Abcd}, \frac{AbCD}{aBcd}, \frac{ABcD}{abCd}, \frac{ABCd}{abcD}.$$

and the other

$$\frac{ABcd}{abCD}, \frac{AbCd}{aBcD}, \frac{AbcD}{aBCd}, \frac{ABCD}{abcd}.$$

Hence it is essential to assume that the sum of the 0, 2 and 4 factor effects is equal to the sum of the 1 and 3 factor effects in estimating recombination from one such set in a four-point linkage test. If this assumption can be made, and there is orthogonality within a set, it can easily be seen that recombination may always be estimated by the addition of the numbers in each mode of gamete formation without risk of bias.

When there are both coupling and repulsion data for every possible pair of factors in a multi-point linkage test, the resulting set of data is

said to be balanced (Fisher and Mather, 1936; Wallace, 1957). In a two-point linkage test, the two possible double heterozygotes are necessary for balance in this sense, and in the three-point test the four possible triple heterozygotes. However, in the four-point test, each of the two sets of four defined above by confounding the four-factor interaction is balanced. Such a set of four may be called a balanced set.

This condition for balance corresponds to the condition that no two-factor interactions should be confounded. The nature of the occurrence of the genotypes in complementary pairs ensures that the

TABLE 1*a*

Genotypes in a 4×4 Latin square

Parental heterozygote	Mode of gamete formation			
	(0)	(1)	(2)	(12)
abc/+++	*abc* 1	*a* *bc*	*c* *ab*	*b* *ac*
+*bc*/*a*++	*a* *bc*	*abc* 1	*b* *ac*	*c* *ab*
ab+/+*b*+	*c* *ab*	*b* *ac*	*abc* 1	*a* *bc*
a+*c*/+*b*+	*b* *ac*	*c* *ab*	*a* *bc*	*abc* 1

main effects are never confounded and so only one parental heterozygote is needed if all the interactions are negligible.

In five- and higher-point data, the problem of selecting balanced sets is complicated and may involve confounding several interactions. A scheme in which balanced sets may be found for higher-order experiments has been devised by Edwards (1958).

3. THE THEORY OF THE χ^2 ANALYSIS

The data from a balanced three-point backcross linkage experiment can be set out as in table 1*a* (*cf.* Parsons, 1957), where a genotype is designated by small letters for the loci at which it is homozygous. The expectations assumed for each observed class are usually taken to be the product of three quantities representing respectively a contribution from the parental heterozygote, a contribution from the mode of gamete formation, and a contribution for the viability of the particular genotype observed. The expectations for the observed classes, corresponding to the genotypes listed in table 1*a*, are given in table 1*b*,

where allowance is made for possible interactions between these three sources of variation. The parameters are defined as follows:—

a_i = proportionate contribution from i^{th} parental heterozygote;
p_j = proportionate contribution from j^{th} mode gamete formation;
v_k = proportionate contribution from the k^{th} genotype;
e_{ijk} = proportionate contribution for the interaction of the k^{th} genotype with the i^{th} heterozygote and the j^{th} mode of gamete formation.

There are thirty-two observed classes, leaving thirty-one degrees of

TABLE 1*b*

Expected proportions corresponding to the genotypes in 1*a*

Parental heterozygote	Mode of gamete formation			
	(0)	(1)	(2)	(12)
abc/+ + +	$a_1p_1v_1e_{111}$ $a_1p_1v_2e_{112}$	$a_1p_2v_3e_{123}$ $a_1p_2v_4e_{124}$	$a_1p_3v_5e_{135}$ $a_1p_3v_6e_{136}$	$a_1p_4v_7e_{147}$ $a_1p_4v_8e_{148}$
+ *bc*/*a*+ +	$a_2p_1v_3e_{213}$ $a_2p_1v_4e_{214}$	$a_2p_2v_1e_{221}$ $a_2p_2v_2e_{222}$	$a_2p_3v_7e_{237}$ $a_2p_3v_8e_{238}$	$a_2p_4v_5e_{245}$ $a_2p_4v_6e_{246}$
ab+/+ + *c*	$a_3p_1v_5e_{315}$ $a_3p_1v_6e_{316}$	$a_3p_2v_7e_{327}$ $a_3p_2v_8e_{328}$	$a_3p_3v_1e_{331}$ $a_3p_3v_2e_{332}$	$a_3p_4v_3e_{343}$ $a_3p_4v_4e_{344}$
a+ *c*/+ *b*+	$a_4p_1v_7e_{417}$ $a_4p_1v_8e_{418}$	$a_4p_2v_5e_{425}$ $a_4p_2v_6e_{426}$	$a_4p_3v_3e_{433}$ $a_4p_3v_4e_{434}$	$a_4p_4v_1e_{441}$ $a_4p_4v_2e_{442}$

freedom after the restriction that the total observed must equal the total expected has been taken into account.

Let us assume that

$$a_i = \tfrac{1}{4}(1+a_i),\ p_j = \tfrac{1}{4}(1+\pi_j),\ v_k = \tfrac{1}{2}(1+\lambda_k) \text{ and } e_{ijk} = 1+\mu_{ijk}.$$

Then the expectation of the observed total from the i^{th} heterozygote, in the j^{th} mode of gamete formation for the k^{th} genotype is

$$m(1+a_i+\pi_j+\lambda_k+\mu_{ijk}+\text{higher-order terms})$$

if it is assumed that a_i, π_j, λ_k and μ_{ijk} are small and $m = N/32$ where N is the observed total. Thus, when the deviations of the proportionate contributions from zero are small, the expectations are approximately linear functions of the parameters.

We have defined four parameters each for the heterozygote and mode of gamete formation effects, and eight parameters for the viabilities or genotype effects. These must be subject to the restrictions

$$\sum_i a_i = 0,\ \sum \pi_j = 0,\ \sum_k \lambda_k = 0 \qquad . \qquad . \quad (3.1)$$

leaving thirteen independent parameters for these effects. The thirty-two parameters μ_{ijk} are subject to the restrictions

$$\sum_{\text{given } i} \mu_{ijk} = 0, \quad \sum_{\text{given } j} \mu_{ijk} = 0, \quad \sum_{\text{given } k} \mu_{ijk} = 0, \quad \sum_{\text{all } ijk} \mu_{ijk} = 0 \quad . \quad (3.2)$$

of which fourteen are independent. This leaves eighteen independent parameters to describe interaction between genotypes, heterozygotes and modes of gamete formation which, together with the thirteen parameters describing the effects discussed above, make up the thirty-one available degrees of freedom.

If values of the parameters are fitted assuming that $e_{ijk} = 1$ for all i, j and k by the method of maximum likelihood, the equations for estimation of the remaining thirteen parameters are given by equating observed row, column and diagonal totals to their expected values. These imply in fact the restrictions 3.2. It is also clear that assuming separate viabilities for the pairs of genotypes in a cell does not affect the estimation of the relative frequencies of the modes of gamete formation.

Now the general procedure followed in applying Fisher's method of scoring (*cf.* Fisher 1956) is as follows. Suppose we make observations on n classes, and the expectation of the r^{th} observation a_r is $M_r(\theta_i \ldots \theta_k)$, where $\theta_1 \ldots \theta_k$ are parameters to be estimated. Then we consider the k linear scores

$$S_i = \sum_{r=1}^{n} a_r \frac{\partial}{\partial \theta_i} (\log M_r) = \sum_{r=1}^{n} K_{ir}\, a_r \text{ say}$$

corresponding to the k parameters, and also the information matrix $I_{ij} = \sum_{r=1}^{n} K_{ir}\, K_{jr}\, M_r$. If now m_r and k_{ir} are the values taken by M_r and K_{ir} when, say, $\theta_1 = 0 = \theta_1 = \ldots = \theta_k$, approximations to the maximum likelihood estimates of $\theta_1 \ldots \theta_k$ are given by the equations $\sum_{j=1}^{k} I_{ij}\, \theta_j = S_i$. If the matrix I_{ij} is diagonal, that is $I_{ij} = 0$ when $i \neq j$, the parameters θ_i may be termed orthogonal and the approximate estimates of them are $\theta_i = S_i/I_i$ where $I_i = I_{ii}$. An approximate test of significance for the departure of θ_i from zero is then given by $\chi_1^2 = S_i^2/I_i$. More accurate estimates and tests are obtained by repeating the scoring procedure with the values of θ_i given above instead of taking all the θ_i to be zero. Now if we assume the state $\theta_i = 0 = \ldots = \theta_1$ corresponds to equality of the expected classes, we have

$$M_r = m[1 + k_{ir}\theta_i + 0\,(\theta^2)]$$

where k_{ir} has the meaning attached to it above and $m = N/n$. Thus k_{ir} give the scores appropriate for a linear system, whatever the values of the θ_i and so scoring at $\theta_i = 0$ is equivalent to assuming a linear system. This is the assumption made in the analysis of variance, where the various effects are made to form a set of orthogonal parameters and

the total sum of squares (S.S.) is split into components which measure the extent of these effects. Now the χ^2 testing departure from equality of the observations a_r is $\chi^2_{n-1} = \Sigma \frac{(a_r - \mathcal{N}/n)^2}{\mathcal{N}/n} = \Sigma \frac{a_r^2}{m} - \mathcal{N} = \frac{1}{m} \times$ S.S. of the a_r. If, therefore, we define the parameters θ_i corresponding to the various effects as for an analysis of variance, we have

$$S_i = \sum_{i=1}^{n} k_{ir} a_r, \quad I_i = \sum_{r=1}^{n} k_{ir}^2 m$$

and the Total S.S. $= \Sigma (a_r - m)^2 = m \sum_{i=1}^{k} S_i^2/I_i +$ Residual S.S. with $n-1-k$ degrees of freedom where $m\, S_i^2/I_i$ is the component of the S.S. for the i^{th} effect, and on division by m this provides the χ^2 detecting the departure of θ_i from zero.

Hence if we carry out an analysis of variance on the data from such a linkage experiment, and divide the component S.S. by m, we obtain an exactly analogous partition of χ^2 for detecting departures from equality of the expected classes. The accuracy of the χ^2 tests thus obtained on the assumed expectations given in table 1*b* depends on the departures from equality being small, so that the expectations are to a good approximation linear functions of the parameters.

The total S.S. for the observations from such a three-point backcross linkage experiment is

$$\Sigma y_{ij1}^2 + \Sigma y_{ij2}^2 - 2n \left[\frac{\Sigma y_{ij1} + \Sigma y_{ij2}}{2n}\right]^2$$

$$= \tfrac{1}{2}\left[\Sigma (y_{ij1} + y_{ij2})^2 - n\left(\frac{\Sigma (y_{ij1} + y_{ij2})}{n}\right)^2\right]$$

$$+ \tfrac{1}{2}\left[\Sigma (y_{ij1} - y_{ij2})^2 - n\left(\frac{\Sigma (y_{ij1} - y_{ij2})}{n}\right)^2\right] + \frac{n}{2}\left[\frac{\Sigma (y_{ij1} - y_{ij2})}{n}\right]^2$$

where y_{ij1} and y_{ij2} are the two observed totals for the complementary genotypes from the i^{th} heterozygote and the j^{th} mode of gamete formation, and $n = 16$. In other words the total S.S. consists of $\frac{1}{2}$[S.S. of the sums of complementary genotypes] $+\frac{1}{2}$[S.S. of the differences of complementary genotypes] $+\frac{1}{2}$ S.S. due to $\Sigma (y_{ij1} - y_{ij2})$. The sums and differences of the complementary genotypes form two 4×4 Latin squares and the S.S. from $\Sigma(y_{ij1} - y_{ij2})$ is the S.S. for the total of the differences square. The "sums" square is the form in which the data are usually presented for the detection of linkage and interference (see Fisher (1949) and Wallace (1957)). The S.S. for the Latin squares can be partitioned in the usual way, namely into components for rows, columns, diagonals and an error term.

If the a_i as defined above are all equal, then the experiment is orthogonally balanced, that is, all the different genotypes are represented equally for each mode of gamete formation. In this case, the

usual treatment effects and interactions represent the corresponding viability effects and interactions of the various loci, as described in the previous section. For example, the S.S. for the two-factor interaction is, symbolically,

$$4\times\tfrac{1}{8}[\{(a-1)\ (b-1)\ (c+1)\}^2+\{(a-1)\ (b+1)\ (c-1)\}^2+\{(a+1)\ (b-1)\ (c-1)\}^2]$$

$$i.e.\ 4\times\tfrac{1}{8}\left[\begin{array}{l}\{(abc+1)+(c+ab)-(a+bc)-(b+ac)\}^2\\+\{(abc+1)+(b+ac)-(c+ab)-(a+bc)\}^2\\+\{(abc+1)+(a+bc)-(b+ac)-(c+ab)\}^2\end{array}\right]$$

$$=4\times\tfrac{1}{8}\left\{4\left[\begin{array}{l}(abc+1)^2\\+(a+bc)^2\\+(b+ac)^2\\+(c+ab)^2\end{array}\right]-\left[\begin{array}{l}(abc+1)\\+(a+bc)\\+(b+ac)\\+(c+ab)\end{array}\right]^2\right\}$$

where *abc* etc. represent the means of the contributions of the corresponding genotypes to the four modes of gamete formation. This is one-half the diagonals S.S. for the " sums " square. In a similar way it can be shown that the diagonals S.S. for the " differences " square is twice that for the main viability effects, and the S.S. for the total of the " differences " square is the S.S. for the three-factor interaction. The expectation of each term in the " differences " square consists only of the difference of the viability contributions from complementary genotypes if the interactions μ_{ijk} are all zero. Hence the S.S. for rows in the " differences " square measures the interaction of viabilities and heterozygotes, and the S.S. for columns, the interactions of viabilities and modes of gamete formation. We therefore obtain the scheme for the analysis of variance given in table 2.

TABLE 2

Scheme for analysis of variance

	d.f.
½ " sums " Latin square	
Parental Heterozygote (rows)	3
Recombination (columns)	3
2-factor interactions (diagonals)	3
Error	6
	15
½ " differences " Latin square	
Viability × Parental heterozygote (rows)	3
Viability × Recombination (columns)	3
Main viability effects (diagonals)	3
Error	6
	15
3-factor interaction	
(S.S. from total of " differences " squares)	1
Total	31

There are in fact 12 degrees of freedom for error, so that only 6 of the 18 degrees of freedom available for describing interactions between the genotypes, the heterozygotes and the modes of gamete formation have been used. These 6 d.f. are made up of 3 d.f. each for the viability × heterozygote and viability × recombination interactions. The remaining unused 12 d.f. would be used to account for recombination × heterozygote and other higher-order interactions. High components for the two " error " terms would indicate the existence of such disturbances.

On division by $m = N/32$ the various components of the analysis of variance will be turned into χ^2s for the corresponding degrees of freedom.

Excluding maternal effects, a viability × heterozygote interaction would indicate a " competition " effect, that is a dependence of the viability of a genotype on the relative proportions of the genotypes with which it is competing. Such " competition " effects may be expected to occur quite generally and to be of considerable importance in the study of population genetics. Haldane (1924) has investigated one form of such an effect occurring in populations, which he has termed " familial selection ". The competition effect would be indicated by the viability × heterozygote interaction, because the different genotypes are represented in differing proportions amongst the offspring of each heterozygote. A balanced linkage experiment may thus be of importance when regarded as a means for detecting viability competition effects due to a number of linked loci.

Recombination × heterozygote interactions are rare, though Parsons (1957) has observed a variation in recombination according to the parental heterozygote which is such an interaction. The occurrence of viability × recombination interactions is most unlikely, as such an effect is almost inconceivable. The interpretation of terms from the " differences " square will be unaltered by significant non-orthogonality as these depend only on the difference of the complementary genotypes, for which the heterozygote contributions must necessarily be equal.

It is clear that a similar analysis of χ^2 can be obtained for any balanced two- or higher-point experiments.

The accuracy of the analysis presented above, which is based on the multiplicative expectations given in table 1*b*, depends on being able to take the expectations as approximately linear expressions in the parameters which is only possible if the effects are small. If, however, logarithms are taken, the expectations are then linear functions of the logarithms of the various parameters. The scores for the logarithms of the parameters are therefore constant, and the information may be expected to vary little with variation in the parameters. It would seem, therefore, that a more accurate analysis could be obtained by doing the calculations on the logarithms of the observed data.

By comparing the analysis obtained before and after taking logarithms it is possible in some cases to distinguish between additive

and multiplicative action of genes. This subject is considered further by Bodmer (1959).

4. APPLICATIONS OF THE ANALYSIS OF χ^2 TO PUBLISHED DATA ON THREE-POINT EXPERIMENTS

(i) *Data of Wallace (1947)*

The data are for a balanced three-point test involving the factors *wavy*-2 (*wv*2), *shaker*-2 (*sh*2) and sex in the house mouse (*Mus musculus*). The analysis of χ^2 is presented in table 3.

TABLE 3

Analysis of χ^2 for Wallace's (1947) data

Latin square of sums	d.f.	S.S.	χ^2	P in %
Rows (parental heterozygotes)	3	57·84	4·09	30 – 20
Columns (recombination)	3	1124·59	79·44	≪0·1
Diagonals (two-factor interactions)	3	5·09	0·36	95 – 90
Error	6	116·19	8·21	30 – 20
	15	1303·71	92·10	≪0·1
Latin square of differences				
Rows (viability × parental heterozygote)	3	62·59	4·42	30 – 20
Columns (viability × recombination)	3	19·34	1·37	80 – 70
Diagonals (main viability effects)	3	1·09	0·08	>99
Error	6	158·69	11·21	10 – 5
	15	241·71	17·08	
Three-factor interaction	1	42·78	3·02	10 – 5
	31	1588·20	112·20	≪0·1

The overall departure from equality of the observed classes is clearly highly significant. By far the largest component is that for recombination, which is, in fact, the only significant component. However, the χ^2s for the two-factor interaction and the main effects are suspiciously small compared with that for the three-factor interaction. The error χ^2_6 in the differences square is also on the verge of significance at 5 per cent. indicating the possible existence of some disturbance, not taken into account in the analysis, as discussed in the previous section. It is clear that, as shown in Wallace's original paper, there is no evidence for non-orthogonality or two-factor viability interactions which might disturb the crude estimates of recombination. The χ^2_3 obtained by Fisher (1949) for the two-factor

interactions by more complicated analysis was 0·43, leaving a deviation χ^2_6 of 7·40, which corresponds with the " error " term of the sums square. These agree well with the results presented in table 3. There is no evidence from the data of any viability × heterozygote interaction or competition effect and, as expected, there is no viability × recombination interaction.

TABLE 4

Analysis of χ^2 for Parsons' (1957) data

Latin square of sums	d.f.	S.S.	χ^2	P in %
Parental heterozygotes	3	14,822	50·3	$\ll$0·1
Recombination	3	2,763,091	9,374·4	$\ll$0·1
Two-factor interactions	3	2,056	7·0	10 – 5
ab-interaction	1	435	1·5	30 – 20
ac-interaction	1	780	2·6	20 – 10
bc-interaction	1	841	2·9	10 – 5
Error	6	24,781	84·0	$\ll$0·1
Total	15	2,804,750	9,515·7	
Latin square of differences				
Viability × parental heterozygote	3	2,121	7·2	10 – 5
Viability × recombination	3	76	0·26	98 – 95
Main viability effects	3	18,928	64·2	$\ll$0·1
main effect of *a*	1	648	2·2	20 – 10
main effect of *b*	1	17,955	60·9	$\ll$0·1
main effect of *c*	1	325	1·1	30 – 20
Error	6	22,997	78·0	$\ll$0·1
Total	15	44,122	149·66	
Three-factor interaction (*abc*)	1	0		
Total	31	2,848,872	9,665·36	$\ll$0·1

(ii) *Data of Parsons (1957)*

These data are for the three loci *scute* (*sc*), *crossveinless* (*cv*) and *vermilion* (*v*) situated on the sex chromosome of *Drosophila melanogaster*. The analysis of χ^2 is presented in table 4.

The departure from equality is again wildly significant, with the main contribution from the recombination effect. As predicted in the last section, the variation in recombination according to parental heterozygote found by Parsons, is indicated by highly significant error terms in both squares. There is a significant departure from orthogonality, as might be expected when the numbers are so large, but this is not severe. The only viability effect is the main effect due to crossveinless, which is highly significant. There is no evidence for any other interactions, although the *bc*-interactions are a little inflated.

The value of χ_3^2 for the two-factor interaction obtained by Parsons (1957) was 5·9 by the usual crude method and 12·6 by Fisher's (1949) method. The value obtained above lies between these two. The residual χ_6^2 of 41·4 given by Parsons is somewhat less than the error terms of either of the Latin squares. However, with such large effects as those indicated for Parsons' data, the theory of the last section would suggest some inaccuracy if scoring is done on the assumption of equal expectations.

Both of these analyses therefore show that the mode of analysis employed in agricultural factorial experimentation gives results in agreement with the analyses using simple χ^2 tests. Some deviation from orthogonality can be tolerated without serious bias as in the *Drosophila* data. Furthermore, this method of calculation has the advantage that all major sources of variation can be detected in one computation.

5. DISCUSSION

The method of analysis of multiple linkage data discussed above enables one to detect and isolate the major sources of variation without much difficulty. The usual model assumed for the estimation of recombination fractions implies no competition effects, and in the case of non-orthogonality may involve heavy computations.

Viability interactions in an orthogonal experiment do not affect estimation of recombination by the addition of the column totals of a Latin square, as they will cancel out (sections 2 and 3). For moderate deviations from orthogonality, with small interactions, as usually occur in practice, addition of the column totals will not result in much error (Parsons, 1957). It seems probable, therefore, that errors in the model which allow for genotypic viability differences are likely to be of the same order as the error in the crude method of estimation obtained by the addition of column totals. Each case must, however, be judged on its own merits.

From the practical point of view, when estimates of recombination are required, it is necessary to do a balanced experiment so allowing for these disturbances. It is clear that deviations from orthogonality should not be too severe.

The analysis of χ^2 provides useful information on viabilities and the form of their interactions associated with major genes which may possibly have important applications in population genetics. A similar method of analysis can be used for other sources of variation associated with major genes. An example would be the fertility and vigour associated with segregating blood-group genes.

6. SUMMARY

1. By applying the principles of the analysis of factorial experiments in agriculture, a comprehensive method of analysing multiple balanced backcross linkage experiments is presented.

2. The technique of confounding provides an interpretation of balance in terms of confounded interactions, and shows what a balanced set as used in multi-point linkage tests implies.

3. It is possible, using this method of analysis, to obtain measures of all the major sources of variation such as the viability effects and interactions, recombination, and competition effects. The implications of performing an analysis on the logarithms of the observed data are briefly discussed.

4. The method is illustrated on data from two balanced three-point experiments.

5. The implication of the method in the estimation of recombination and other possible applications are discussed.

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MULTIPLICATIVE EFFECTS AND THE LOGARITHMIC TRANSFORMATION IN THE ANALYSIS OF BALANCED MULTI-POINT LINKAGE TESTS

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1. INTRODUCTION

In a previous paper Bodmer and Parsons (1959) have outlined a comprehensive analysis of χ^2 for data from balanced multi-point linkage tests. By drawing an analogy with factorial experimentation, it was possible to obtain components measuring genic viability effects and interactions, recombination effects, parental heterozygote effects and possible interactions between these three sources of variation. These components were obtained from a corresponding analysis of variance on the assumption that, at least to a good approximation, the various effects were additive, whereas the usual treatment assumes a multiplicative set of effects (Fisher, 1949; Parsons, 1957). If these are large, departure from additivity may be severe and should be detectable in a difference between the interactions as measured on an additive or a multiplicative scale.

If we take logarithms, we can turn a multiplicative system into an additive system involving the logarithms of the original effects. It will be shown that a similar analysis to that given by Bodmer and Parsons, but based on the logarithms of the observed data provides, to a good approximation, measures of the various effects and interactions on a multiplicative scale. This makes it possible to detect differences in the mode of gene action, at least on viability, from data obtained in a balanced multi-point linkage test.

2. THE LOGARITHMIC TRANSFORMATION

The expectation of the observed frequency in a balanced three-point test of the k^{th} genotype from the i^{th} parental heterozygote and the j^{th} mode of gamete formation may be taken as $a_i\, p_j\, v_k\, e_{ijk}$ (see table 1, Bodmer and Parsons (1959)). The parental heterozygote, recombination and genotypic effects are a_i, p_j and v_k respectively and e_{ijk} is the interaction between these effects. The logarithm of the expectation is therefore the sum of the logarithms of the multiplicative effects. In order to be able to interpret an analysis of variance and so of χ^2 on the logarithms of the observed data, the logarithms of these parameters must satisfy restrictions of the form considered by Bodmer and Parsons for the additive effects.

Consider the transformation of the proportions a_i given by

$$b_i = a_i \,/\, [\pi a_i]^{\frac{1}{2}}.$$

It is clear that $\pi b_i = 1$ and so $\sum_i \log b_i = 0$. Furthermore, when all the a_i are equal $b_i = 1$ and so $\log b_i = 0$. Hence, if we transform p_j to a_j and v_k to w_k by similar transformations, the sums of the logarithms of these new parameters are zero. Since e_{ijk} are not proportions which must add up to unity, the form of restriction imposed on them is immaterial. The restrictions appropriate for the multiplicative model are

$$\underset{\text{given } i}{\pi e_{ijk}} = 1, \quad \underset{\text{given } j}{\pi e_{ijk}} = 1, \quad \underset{\text{given } k}{\pi e_{ijk}} = 1, \quad \underset{\text{all } i,j,k}{\pi e_{ijk}} = 1.$$

The condition that the total observed should equal the total expected, is replaced by the condition that the sum of the logarithms of the observed frequencies should equal the sum of the logarithms of the expected frequencies. It may be noted that these conditions are analogous to calculating a S.S. in an analysis of variance, given the mean of the observations, and provide ancillary statistics indicating a measure of the amount of informatio.. contained in the sample.

The component S.S. for the analysis of variance on the logarithmic data will be of the form

$$\left[\sum_{r=1}^{n} k_{ir} \log a_r\right]^2 \Big| \sum_{r=1}^{n} k_{ir}^2$$

where $a_1 \ldots a_n$ are the observed frequencies and $\sum_{r=1}^{n} k_{ir} \log a_r$ is approximately the score for the i^{th} multiplicative effect. The corresponding information is given approximately by the variance of the score (see *e.g.* Fisher (1956)), *i.e.* by

$$v\left[\sum_{r=1}^{n} k_{ir} \log a_r\right] \simeq \sum_{r=1}^{n} k_{ir}^2 \, v[\log a_r] \simeq \sum_{r=1}^{n} k_{ir}^2 \frac{1}{m^2} v(a_r) \simeq \frac{1}{m} \sum_{r=1}^{n} k_{ir}^2$$

where scoring is at equal expectations of the observed classes so that $v(a_r) \simeq m$ the overall mean. Hence if we multiply the components in the analysis of variance by m, or $m\,(\log_e 10)^2$ if the calculations were done on logarithms to the base ten, we obtain the corresponding χ^2s. The score for any effect is unaltered by variation in the other effects, and the information may be expected to vary only by a small amount. This analysis should therefore provide reliable measures for multiplicative effects of any magnitude.

3. MULTIPLICATIVE EFFECTS AND INTERACTIONS

It is possible to define effects and interactions for a multiplicative system which are analogous to those defined for an additive system. Thus, in terms of logarithms, the three-factor interaction, for example, is

$$\tfrac{1}{4}[\log abc + \log a + \log b + \log c - \log 1 - \log bc - \log ac - \log ab]$$

$$= \log \sqrt[4]{\frac{abc \,.\, a \,.\, b \,.\, c}{1 \,.\, bc \,.\, ac \,.\, ab}}.$$

Hence we may define $4\sqrt{\frac{abc \,.\, a \,.\, b \,.\, c}{1 \,.\, bc \,.\, ac \,.\, ab}}$ as a consistent measure of three-factor interaction on a multiplicative scale. The principles of confounding may also be applied, the ratios of the sets of confounded treatments being indistinguishable from the block effects instead of the differences.

It may be noted that for a multiplicative system estimation by geometric means replaces estimation by arithmetic means. This implies, for example, that in the model for a three-point test considered above, when all the interactions $e_{ijk} = 1$, consistent estimates of the various effects can be obtained by equating observed to expected. The estimates will be simple functions of the observed frequencies, whose variances can be calculated by Fisher's approximate formula (Fisher, 1925-54), and may be expected to be fairly efficient.

When performing an analysis on the logarithms of the observed data it is the multiplicative effects and interactions, as considered above, which are being tested. There is thus a duality between additive and multiplicative systems, the experimental techniques for the one being applicable to the other.

4. NUMERICAL ILLUSTRATIONS OF THE ANALYSIS OF χ^2 WITH THE LOGARITHMIC TRANSFORMATION

In order to illustrate the method of analysis and the possibility of detecting differences between additive and multiplicative gene action an example is given of the analysis applied to an artificially constructed set of data for a two-point experiment.

The form of the analysis of variance for a two-point experiment can be obtained exactly as that for a three-point experiment was obtained by Bodmer and Parsons (1959). The data consist of observed frequencies from four pairs of complementary genotypes, which can be arranged in a 2×2 Latin square. From these are obtained the " sums " and " differences " squares, giving the scheme for the analysis of a two-point test shown in table 1, where a and b are the two factors concerned.

A recombination fraction of 20 per cent. was assumed and the relative contributions of coupling and repulsion heterozygotes were taken to be 9 : 8. Viabilities were assumed which showed a competition effect, and the expected proportions for each class calculated as the product of the relevant parameters. The set of data was obtained by multiplying the expected proportions by a convenient observed total. The assumed values of the parameters and resulting set of data are given in table 2.

The competition effect is such as might be expected with additive viabilities and one genotype considerably deficient in the coupling phase when there is severe competition from the non-recombinant normals. The viability of the b genotype is considerably improved

in the repulsion phase when the normals are in the less frequent recombinant category, and that of the severely deficient genotype *ab*

TABLE 1

Scheme for the analysis of a two-point experiment

	d.f.
½ " sums " Latin square	
Parental heterozygotes (rows)	1
Recombination (columns)	1
Two-factor interaction (diagonals)	1
	3
½ " differences " Latin square	
Viability × Parental heterozygote (rows)	1
Viability × Recombination (columns)	1
Main viability effect of *a* (diagonals)	1
	3
½ total of " differences " square	
Main viability effect of *b*	1
	7

TABLE 2

Artificially constructed set of data for a two-point experiment

Viabilities in	Genotypes				
	1	*ab*	*a*	*b*	
Coupling	10	2	8	5	*25*
Repulsion	10	3	9	8	*30*

Recombination 20 per cent.
Representation of heterozygotes: Coupling : Repulsion = 9 : 8

	Non-Recombinants		Recombinants		Total
Coupling	1	432	*ab*	87	
	ab	87	*b*	54	
Repulsion	*a*	288	1	81	
	b	255	*ab*	24	
					1308

is also improved a little. It should be noticed that both sets of viabilities for coupling and repulsion show considerable multiplicative interaction but only mild additive interaction.

The χ^2 analysis on the original and the logarithmic data is given in table 3. As expected, the two-factor interaction is significant only on the logarithmic data, showing multiplicative but not additive interaction of the two gene effects on viability. The analysis on the original data shows a highly significant viability × heterozygote interaction or competition effect, but indicates a viability × recombination interaction of the same magnitude. It should be pointed out that in the analysis

TABLE 3

Analysis of χ^2 for the two-point data of table 2

Latin square of sums	d.f.	χ^2s	
		Original data	Logarithmic data
Heterozygotes	1	0·11	0·95
Recombination	1	509·1	666·6
Two-factor interaction	1	2·75	49·2
	3		
Viability × Heterozygote (competition effect)	1	63·4	11·2
Viability × Recombination	1	63·4	0·09
Main viability effect of *a*	1	86·3	100·7
	3		
Main viability effect of *b*	1	167·4	238·7
	7		

of a two-point experiment a competition effect may inflate the viability × recombination component, but that the reverse is unlikely to occur. It is interesting to note that on neither analysis is the slight departure from orthogonality detected. The exceedingly low value for the viability × recombination effect on the logarithmic analysis is because the actual expected values were used for the data and so no random variation has been allowed for. The known situation is seen to be clearly and accurately represented by the analysis on the logarithmic data.

The usual heterogeneity χ^2_1, calculated from the 2×2 Latin square of sums to detect viability interactions, is 5·7011. This considerably underestimates the significance of the multiplicative interaction, and it is to be expected, in general, that the accuracy of such a χ^2_1 in detecting multiplicative interactions lies between those obtained from an analysis of χ^2 on the original and the logarithmic data. The estimate of recombination obtained, using the product formula of estimation

(Fisher, 1925-54), is $0{\cdot}1865 \pm 0{\cdot}01$. Thus the effect of viability competition on the usual method of estimation is appreciable though the estimate obtained is not significantly different from the true value of 20 per cent. This effect may be expected to decrease as the number of loci used increases.

An analysis on the logarithms of Wallace's (1947) data for a balanced three-point test involving the factors *wavy*-2 (*wv*-2), *shaker*-2

TABLE 4

Analysis of χ^2 on Wallace's (1947) data after taking logarithms

Latin square of sums	d.f.	χ^2	P in %
Parental heterozygote	3	5·63	20 – 10
Recombination	3	79·34	
Two-factor interactions	3	0·98	70 – 50
Error	6	8·20	30 – 20
	15		
Latin square of differences			
Viability × parental heterozygotes	3	2·36	50
Viability × recombination	3	4·88	20 – 10
Main viability effects	3	0·13	99 – 98
Error	6	16·11	2 – 1
	15		
Three-factor interaction	1	2·30	20 – 10
	31		

(*sh*-2) and sex in the house mouse is given in table 4. The analysis is on the whole very similar to that given by Bodmer and Parsons (1959) on the original data. None of the interaction components show significant increases, showing that the effects are too small for a difference between additive and multiplicative scales to be detected. The only striking difference is the significant component for the error term from the differences square. This is, however, the largest of eight χ^2s, not including the component for recombination, but taking this into account the significance level is still less than 10 per cent. This supports the suspicion mentioned by Bodmer and Parsons (1959) that there exists some disturbance not taken into account by an analysis of the above form. A closer examination of the original data indicates a disturbance of the ratio of the complementary genotypes $+sh_2Y.$ and $wv_2+X.$ from the four parental heterozygotes, the other complementary pairs being unaffected. A χ^2_3 of 11·6 is obtained with a

probability of less than 1 per cent. This seems to confirm the above suspicion, as such an effect cannot be explained simply in terms of a competition effect or straightforward viability interaction. As the disturbance is only indicated by the differences square it cannot affect the estimation of recombination.

5. DISCUSSION

As with any χ^2 test, small numbers of observations render the test inaccurate. Moreover large disturbances can arise from the differences of the logarithms of small numbers so that an analysis on the logarithmic data is more sensitive to disturbances due to a small number of observations. In order to take this into account some form of weighted analysis, using the number of observations, or amount of information, as weights, would be required.

Clearly, for it to be possible in practice to detect differences between additive and multiplicative action of genes, the effects must be fairly large or otherwise the two modes of action are approximately equivalent. In general three- and higher-point experiments will prove very difficult to run when there are severe viability disturbances. Hence differences in the mode of gene action are most likely to be detected with two-point experiments where more severe disturbances can be tolerated. This is well illustrated by preliminary results from a two-point back-cross linkage experiment with the factors *pallid* and *fidget* in the house mouse, being carried out by the author. Both these factors cause severe reduction in viability, and the results so far obtained show clearly multiplicative, as opposed to additive, action of the genes concerned on viability. Similar results have been observed by Parsons (1959) with two-point experiments in *Drosophila melanogaster*.

Whether viability effects of genes are additive or multiplicative will in general depend on the detailed physiological and biochemical situation. For example, genes with some sort of dosage effect may be expected to act additively. On the whole it seems more reasonable to suppose that the viability effects of genes act in a multiplicative way, the effects on the Malthusian parameter, as defined by Fisher (1930), being therefore additive. However, there is, at present, little data which could justify such an assumption.

The transformation to logarithms employed above is an example of one of the many transformations that are used in statistical analysis for diverse reasons. In this situation it is in fact employed to turn the multiplicative system into an additive system to which the standard techniques of analysis can be applied. A distinct meaning can be attached to the transformation as used here and a similar process could be carried out to investigate the possibility of gene action being a mixture of the multiplicative and the additive as might, in general, be expected to occur. It would seem, however, that in general due consideration must be given to the meaning of a transformation before

results from an analysis of transformed data can be legitimately interpreted.

6. SUMMARY

1. The use of the logarithmic transformation in the analysis of a multiplicative system of effects is described.

2. Consistent measures of multiplicative effects and interactions are defined and the duality between multiplicative and additive systems is discussed.

3. The methods of Bodmer and Parsons (1959) are applied, in conjunction with the logarithmic transformation, to the analysis of data from backcross linkage experiments.

4. The possibility of detecting differences between multiplicative and additive gene action on viability in data from such experiments is discussed.

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GENETIC STUDIES OF THE SYRIAN HAMSTER

II. PARTIAL ALBINISM

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1. INTRODUCTION

THE present account of investigations with the Syrian hamster (*Mesocricetus auratus*) is a continuation of work reported earlier (Robinson, 1958*b*). The search for possible genetic linkage between the various colour mutants has been extended to include a new recessive allele (partial albinism or the " dark-eared albino ") which was imported from the United States in 1955 (Robinson, 1957).

2. DESCRIPTION OF MUTANT ALLELES

A detailed description of the fur pigmentation of the normal Syrian hamster, and of the phenotypes engendered by the mutant alleles cream, ruby-eye and piebald, has been presented in the earlier paper. The following consists of new observations.

(i) *Cream*

The cream phenotype (symbol *e*) is produced by an inhibition of melanic pigmentation. The body fur is rich cream-yellow or yellow-orange. There is considerable variation between animals, much of which is determined by independent polygenes. The vestiges of melanin which remain upon the animal are confined to the eye, the dermis of ear pinnæ, the skin of the hip-gland and in the perineum. The development of this pigment in the skin is regular and corresponds to that present in the skin of the normal animal. In many crosses, however, there is a disturbance of melanin development in the pinnæ. The pigment appears to be laid down patchily if the erect pinnæ are viewed against a strong light. This condition of the ears has been termed " marbled " and is usually a transient stage to more or less normal pigmentation. In some creams, however, the marbled condition persists for a long period.

(ii) *Ruby-eye dilute*

The expression of ruby-eye (*ru*) is that of a typical eye and coat colour dilution. The pupil of the eye glows ruby in reflected light and the body fur is bluish-fawn. The male ruby-eye is either completely sterile or transiently fertile. Preliminary observations on this question have been given in the first paper. A more complete analysis on the sterility onset in the male has been communicated by Bruce (1958).

The ruby-eye hamster is less viable than the normal. The impaired vigour is apparent throughout life, being especially noticeable at parturition. The parturition death rate for the ruby-eye female is appreciably higher than that for the normal. The overall viability from the combined data of the previous paper at 21 days of age for *ruru* young is $0{\cdot}5781 \pm 0{\cdot}0408$. The comparable viabilities for the two crosses of this paper are $0{\cdot}5919 \pm 0{\cdot}0641$ and $0{\cdot}6178 \pm 0{\cdot}495$ respectively, and are in general agreement. The previous data did not reveal a significant difference of viability between the sexes ($0{\cdot}0324 \pm 0{\cdot}0833$). However, the present data reveal a significant difference (table 1). The mean difference is $0{\cdot}1621 \pm 0{\cdot}0527$ in favour of the female and is highly significant. In spite of the curiously discordant value of cross III for males, the five groups appear to be homogenous.

TABLE 1

Significance of sex differences of viability of ruby-eye hamsters at 21 days

Cross	♂	♀	Difference
I	0·4588	0·5077	$-0{\cdot}0489 \pm 0{\cdot}1359$
II	0·5000	0·6106	$-0{\cdot}1106 \pm 0{\cdot}1246$
III	0·8833	0·6853	$0{\cdot}1980 \pm 0{\cdot}1971$
IV	0·4768	0·7066	$-0{\cdot}2298 \pm 0{\cdot}0934$*
V	0·4824	0·7533	$-0{\cdot}2709 \pm 0{\cdot}0996$†

* significant † highly significant

The small inter-group differences yield an insignificant heterogeneity of $\chi^2_4 = 5{\cdot}61$ ($P > 0{\cdot}20$). Except for the aforesaid value, it is of interest to note that the viabilities for both sexes display a consistent rising trend. It is suggestive that the viability of ruby-eye is slowly improving through succeeding generations—the female at a significantly faster rate than the male. As part of the segregation data, the male *ruru* is a crossover class in the joint segregation of *ru* and *Y* (see next section), and it is conceivable that the shortage could be due to linkage between *ru* and *Y*, the male-sex differentiating segment of chromosome. Inspection of the complete segregations (table 7, columns 2 and 3) do not support the contention. The two complementary non-ruby-eye classes are not unbalanced.

The difference in relative viability of the sexes for ruby-eye is intriguing since a similar difference cannot be detected for piebald. The viability of piebald is much less than that of ruby-eye and, if the dissimilar sex viability is a consequence of an influence common to males (uterine environment), a similar effect should be patent for the piebald. This is not so, and it may be inferred, consequently, that the *ru* allele is sexually differentiated for some of its effects. This may take the form of sterility and probably reduced general vigour in the male. This may be obscured in practical breeding to some extent by the

extra stresses to which females are exposed from successive parturitions and lactations.

(iii) *Piebald*

The piebald (*s*) is an irregular form of recessive white spotting. Low-grade animals have the white pattern restricted to the foreface and breast while high-grade animals have extensive white areas on the body. Selective breeding has produced almost completely white specimens with small spots of coloured fur in the regions of the eye and ears and upon the hind quarters. In general, the progression of white is an anterio-posterior direction with a tendency for a post-medial belt of white.

Animals homozygous for piebald are of reduced general stamina. At the recording age of 21 days the estimated viability is approximately 50 per cent. of the normal hamster. These losses are mainly attributable to intra-uterine death precipitated by (*a*) embryonic or fœtal competition between normal and piebald, in which the latter succumb, and (*b*) developmental defects of the urino-genital system (see Orsini, 1952; Bock, 1953; and Foote, 1955). The defective development may extend to the vertebral column since the piebald individual may occasionally possess a bent or twisted tail. A piebald male, with a bent tail, was mated to two different non-piebald females, each having normal tails. The F_1 were normal coloured and with normal, straight tails, numbering 14 and 15 young, respectively. The F_2 of the first female segregated into 39 normal and 7 piebald, two of the latter having bent tails. The F_1 of the second female segregated into 38 normal and 7 piebald, one of the latter with a bent tail. The viability of piebald for the combined data is $0{\cdot}5455 \pm 0{\cdot}0501$, not differing from expectation. The association of bent tail with the presence of piebald is highly significant, $\chi_c^2 = 11{\cdot}06$ ($P < 0{\cdot}01$; with Yates' correction). It is conceivable, of course, that the occurrence of bent tail is due to developmental and parturitional hazards but this does not explain the association with piebald. An alternative explanation postulating a gene linked to piebald would require weak penetrance of the "bent tail" gene, in view of the low incidence of individuals with the abnormal tail vertebrae.

In view of the difference between the viability of the sexes for ruby-eye animals it is pertinent to enquire if such a difference may exist for the piebald. Crosses II and III showed a non-significant lower viability for the male, but in the present cross IV the tendency is reversed. The viability of the male sex is $0{\cdot}3768 \pm 0{\cdot}0784$ and that for the female, $0{\cdot}2691 \pm 0{\cdot}0628$. Combining differences between viabilities for the three segregations gives the insignificant mean difference of $0{\cdot}0451 \pm 0{\cdot}0621$ in preference of the female. It may be mentioned that the differences were found from overall viabilities, which are lower than those given previously where an attempt was made to obtain estimates unbiased by the *ruruss* interaction.

(iv) Partial albinism

A mutation to an albinotic allele occurred in the United States, *circa* 1951-52. Adult animals, homozygous for the allele, have completely white fur and pink eyes but with pigmented ears. Pigment may form in certain regions of the skin in the fully mature animal, in the prepuce and scrotum of males and occasionally, not invariably, in the perineum of females. No pigment has been observed in the hip-gland. The intensity of pinnæ pigmentation eventually attains that present in the normal but the amount in the genitals is reduced.

The young albinotic hamster at 21 days of age is completely devoid of pigment. By 26 days of age, however, the ear pinnæ are distinctly tinged with pale grey, especially along the edges. By the thirty-first day the pinnæ are suffused grey. The rate of pigment formation is initially rapid but slackens later. The age range for onset of ear pigmentation is from 24-28 days while the age range for the ear to qualify as suffused grey varied from 27-36 days. A correlation was noticed between early onset of the grey tinge and a rapid transition to suffused grey. While a correlation diagram portrayed a path of clustered entries about a regression line of slope, $b = 0{\cdot}1360 \pm 0{\cdot}0956$, the regression coefficient is subsignificant for a sample of 40 individuals. The eye colour of the young dark-eared albino is the light pink of a true albino. The eye colour of an adult animal is also that of complete albino, but some individuals appeared to have a ruby tint. The possibility that a few animals may have some pigment in the deeper layers of the eye should be recognised.

These observations strongly suggest that the present mutant of the hamster is that of a thermo-sensitive allele of the albino locus, comparable to the allele primarily responsible for the phenotype of the "albino" guinea-pig, Himalayan rabbit or Siamese cat. For this reason the symbol proposed for the allele is c^a. It will be noted that other extremities of the body in the hamster, the nose, feet and tail, are not pigmented as is the case for the rabbit and cat. The three alleles of the guinea-pig (c^a), rabbit (c^h) and cat (c^s) differ in the degree of pigment formation. The Siamese cat allele allows more pigment to be formed than is usually found in either the "albino" guinea-pig or Himalayan rabbit. The Siamese cat usually has pigment in the dorso-lateral regions. In the guinea-pig and rabbit this is rare, while in the hamster it is unknown. It is probable that the hamster allele c^a permits less pigment to manifest than the respective alleles of the rabbit and cat. This comparison assumes, of course, the usual (*i.e.* most frequent) polygenic background for pigment expression of the mutant for each species. Additional evidence for exact placing of partial albinism may be found from the effect of the homologous alleles upon the development of yellow pigment. The cat gene allows some yellow pigment upon the extremities (the red-pointed Siamese) while the guinea-pig (Wright, 1915), rabbit (Robinson, 1958*a*, pp. 268-269) and hamster alleles do not. Ranking the four mutants for amount

of pigmentation typically expressed, gives the probable order $c^s > c^h > c^d > c^a$.

It should perhaps be mentioned that the fullest expression of himalayanism is dependent upon the combination of the albinistic allele with non-agouti (*a*). Thus, the Himalayan rabbit is of genotype *aac*^{h}*c*^{h}. This mutant allele is unknown in the hamster. The appearance of non-agouti in the hamster would probably result in a more typically acromelanic phenotype. Black pigment is present in the ears but not on the legs or tail, and this is probably the reason why the ears only are coloured. The amount of melanic pigmentation formed upon the more exposed, cooler parts of the body (ear pinnæ, prepuce and scrotum) is comparable to that found in the normal phenotype. It would be desirable if some direct experiments could be performed to establish the nature of the differential thermal thresholds for various sectors of the body. This has been accomplished by Iljin and Iljin (1930) for the cat and by numerous experiments for the rabbit (Robinson, 1958*a*, pp. 240-246).

The gene for partial albinism is epistatic to most of the other colour mutants; only when the usual dark ear colour is modified may the presence of another mutant allele be assumed. The *e* allele usually has no effect upon ear colour and, consequently, animals of genotype *c*^{d}*c*^{d}*E*— and *c*^{d}*c*^{d}*ee* are indistinguishable. This is not invariably the case, however, for some *ee* hamsters display a tardy development of ear melanism, the "marble" ear. This is paralleled by some *c*^{d}*c*^{d}*ee* animals, in which event the ear colour tends towards a patchwork grey. Three of these "grey-eared albinos" were tested by mating to cream. The resulting litters consisted of twenty-one young, all cream. It is unfortunate that the expression of greyness is too variable for reliable identification of the genotype *c*^{d}*c*^{d}*ee*. Prepuce and scrotal pigmentation are not affected by *e* and both the dark-eared albino (*c*^{d}*c*^{d}*E*— or *c*^{d}*c*^{d}*ee*) and the grey-eared albino (*c*^{d}*c*^{d}*ee*) display similar genital pigment. The ear colour of the ruby-eye hamster is peach-grey, produced by a sharp reduction in skin pigmentation throughout the whole body. In combination with *c*^{d}, the reduction appears to be enhanced. The genotype *c*^{d}*c*^{d}*ruru* is a "synthetic albino" with almost flesh-coloured ears and genitals. The ear colouring varies from clear pink to peach-grey but not attaining the degree of peach-grey found in the non-albinotic ruby-eye. The reason for this is not obvious, but it is suggestive that the thermal threshold of melanin production in *c*^{d}*c*^{d}*ruru* animals may be higher than that in *c*^{d}*c*^{d}*Ru*—. The genotype *c*^{d}*c*^{d}*eeruru* is also a synthetic albino.

Partial albino homozygous for piebald (*c*^{d}*c*^{d}*ss*) may occasionally reveal the genotype by the presence of flesh-coloured spots on the ears. Since the ear pinnæ are often unspotted, even in high-grade piebalds, this is an unreliable guide. The steep reduction in body weight could be used as an indicator of homozygosity of piebald. At 21 days of age the mean weight of piebalds is approximately 77 per cent. of that of

non-piebald. These two features may discriminate the piebald albino in special instances but perhaps not with sufficient accuracy for critical segregation analysis.

It has been observed that the various mutants appear to be associated with small differences of temperament. The dark-eared albino appears to be neutral in this respect. Defining the normal as standard, the cream is a more placid animal (on the average), while the ruby-eye and piebald are more nervous. The piebald is more irascible than the ruby-eye. The roster would be, therefore, for increasing nervousness: cream $<$ normal $\simeq$ albino $<$ ruby-eye $<$ piebald. Finally, in the crosses so far completed, the c^d allele appears to be fully penetrant, and individuals homozygous for it, of normal viability.

TABLE 2

Data from experimental crosses

Cross	Type	*CERu*		*CeRu*		*CEru*		*Ceru*		c^dRu		c^dru	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
IV	*YERusc^d/XeruSC* × *YERusc^dXeruSC*	*S* 136	137	38	43	27	36	6	7	59	64	7	13
		s 17	10	8	5	1	5	0	0	♂		♀	
V	*YERuc^d/XeruC* × *YERuc^d/XeruC*	346	348	108	106	45	82	28	32	172		209	

3. ANALYSIS OF SIMULTANEOUS SEGREGATION

Two series of matings have been completed involving the joint segregation of c^d with *e*, *ru* and *s*. The results of the two crosses (IV and V) are summarised by tables 2 and 3. The segregation of cross IV is partitioned as rows into normal (*S*) and piebald (*s*) as combined with the genotypes heading the columns. In addition to the gene symbols proposed above, the symbols *Y* and *X* are employed as representing the differential segments of the sex chromosomes. The segregation of a mutant allele may display a sex association which could be interpreted as partial sex-linkage. For analytical convenience, *Y* is treated as a dominant gene. Where two dominant genes enter the cross from the same parent, this is taken as coupling association. All the young were sexed and classified for coat colour at 21 days, except for the identification of c^dc^dRu- and c^dc^druru, where the ear colour distinction was deferred to between 40 and 50 days of age. Unfortunately, this separation of the two albinotic types could not be made for cross V. Estimation of the parameters for the theoretical genotypes underlying the observed segregation is achieved by maximum likelihood scores. Iterative correction factors were applied until the parameters converged sufficiently. The required variances were found by inversion

of the respective information matrices. The inviability which accompanies the segregation of *ru* and *s* is symbolised by *v* and *u*, respectively, and the crossover value by *p*.

TABLE 3

Classification of data for segregation analysis

Cross	Mating Type	Loci		Phenotypic classes				Total
		a	*b*	*AB*	*aB*	*Ab*	*ab*	
IV	CII	*e*	*ru*	300	94	69	13	*476*
	RII	*e*	*s*	336	94	73	13	*476*
	RII	*e*	c^d	369	107	143		*619*
	CIB	*e*	Y	181	42	188	65	*476*
	RII	*ru*	*s*	354	76	40	6	*476*
	RII	*ru*	c^d	394	82	123	20	*619*
	CIB	*ru*	Y	258	41	259	61	*619*
	CII	*s*	c^d	430	46	143		*619*
	RIB	*s*	Y	207	26	223	20	*476*
	RIB	c^d	Y	233	66	243	77	*619*
V	CII	*e*	*ru*	694	127	214	60	*1095*
	RII	*e*	c^d	821	274	381		*1476*
	CIB	*e*	Y	391	136	430	138	*1095*
	RII	*ru*	c^d	908	187	381		*1476*
	CIB	*ru*	Y	454	73	454	114	*1095*
	RIB	c^d	Y	527	172	568	209	*1476*

C = coupling, R = repulsion, I = intercross, B = backcross, for the respective alleles.

(i) Cross IV

This segregation is an F_2 generation from $YXEERuRussc^dc^d \times XXeeruruSSCC$. The regular segregation ratios display considerable disturbance and inspection of table 2 reveals a deficiency of both *ruru* and *ss* zygotes.

	χ^2	d.f.	*P*
e segregation . . .	1·61	1	>0·20
ru ,, . . .	23·98	1	<0·001
s ,, . . .	62·19	1	<0·001
c^d ,, . . .	1·19	1	>0·20
sex ,, . . .	0·71	1	>0·30

Estimation of the recombination fraction for the ten possible gene-pair comparisons is complicated by the impaired viability of segregants homozygous for *ru* and *s*. The three comparisons $e-c^d$, $e-Y$ and c^d-Y, have the inviability apportioned over complementary crossover and non-crossover classes and estimation is straightforward. For the

remaining comparisons a simultaneous estimation with either v or u is unavoidable.

Simultaneous segregation of c^d and s is marred by the inviability of ss segregants and by the epistasis of c^d to s. The probabilities of the three classes are as follows ($P = (1-p)^2$), with the values of $p = 0{\cdot}4036 \pm 0{\cdot}0946$ and $u = 0{\cdot}3912 \pm 0{\cdot}0355$ as the solution of the derived maximum likelihood functions.

	CS	Cs	c^dc^d
Expectation . .	$\frac{2+P}{3+u}$	$\frac{u(1-P)}{3+u}$	$\frac{1-P+uP}{3+u}$
Observation . .	430	46	143

Examination of previous data upon the joint segregation of *ru* and *s* has suggested that the inviability of each may interact in the double recessive *ruruss*. These animals are very feeble, their weight at 21 days is 45 per cent. of normal, and their prenatal death rate may be greater than that of a simple compound of the prenatal rate for *ruru* and *ss* alone. Representing x as the viability of *ruruss* (chance of survival of the phenotype = *xvu*), the observed segregation would be produced by the following values $v = 0{\cdot}6441 \pm 0{\cdot}0814$, $u = 0{\cdot}3390 \pm 0{\cdot}0565$ and $x = 0{\cdot}6987 \pm 0{\cdot}3184$; x does not differ significantly from unity. The method of calculation of x and its sampling variance is given in the preceding paper, where x is significant. However, in all of the data to hand, the *ruruss* individuals constitute a crossover class. In consequence it has not been possible to decide if the shortage in this class is due (*a*) to the inviability interaction or (*b*) to linkage between *ru* and *s*.

(ii) Cross V

The second cross is similar to the first, but without piebald, and the resultant F_2 generation is shown by tables 2 and 3. Statistical examination of the single gene ratios disclosed the usual deficiency of *ruru* individuals. There is also a deficiency of males which just exceeds the conventional 5 per cent. level of significance. This may be a chance deviation but the tests for a sex association between the mutants indicate that the deficiency is concentrated in the phenotypic class *YXruru*. The male ruby-eye is a crossover class in the *ru*-*Y* comparison and the recombination fraction computed is significant ($\chi^2_1 = 5{\cdot}84$; table 7, line 5). That the association is not due to genetic linkage with the *Y* segment of chromosome is shown by the same table. The two complementary non-ruby-eye classes are of exact equality which is scarcely to be expected with linkage. The association between *ru* and *Y* revealed by the combined estimate of p, which just attains the

border-line of significance ($\chi_1^2 = 3{\cdot}85$, table 7, total), is probably due to the excess mortality of *ruru* males.

	χ^2	d.f.	P
e segregation . .	0·0003	1	>0·98
ru ,, . .	36·65	1	<0·01
c^d ,, . .	0·52	1	>0·30
sex ,, . .	4·12	1	<0·05

The outcome of tests for possible linkage between the six gene-pair comparisons are tabulated in table 4. The assessment of independent segregation for the pair $ru-c^d$ is complicated by epistasis of c^d to *ru*

TABLE 4

Estimated recombination fractions (p) *and viabilities* (v *or* u)

Cross	Loci	p	v or u
IV	$e-ru$	0·5477±0·0386	0·6244±0·0578
	$e-s$	0·5640±0·0447	0·3209±0·0498
	$e-c^d$	0·5756±0·0595	
	$e-Y$	0·4349±0·0397	
	$ru-s$	0·4544±0·0578	v 0·5933±0·0854; u 0·3128±0·0508
	$ru-c^d$	0·4786±0·0343	0·5919±0·0641
	$ru-Y$	0·4383±0·0396	0·5919±0·0641
	$s-c^d$	0·4036±0·0946	0·3912±0·0355
	$s-Y$	0·4396±0·0516	0·3209±0·0498
	c^d-Y	0·5186±0·0348	
V	$e-ru$	♂ 0·4062±0·0392 *	0·4824±0·0608
		♀ 0·4635±0·0338	0·7533±0·0789
	$e-c^d$	0·4993±0·0393	
	$e-Y$	0·5151±0.0262	
	$ru-c^d$	0.4305±0.0915	0·5525±0·0827
	$ru-Y$	0·4288±0·0295 *	0·6178±0·0495
	c^d-Y	0·5237±0·0225	

* Significant, see text.

and the lower inviability of *ru*. Three phenotypic classes can be distinguished and the theoretical probabilities of these are as shown for the $s-c^d$ comparison of cross IV, with v substituted for u and $P = p^2$. The values of p and u satisfying the maximum likelihood functions are 0·4305±0·0915 and 0·5525±0·0827, respectively. The analysis for random segregation of the genes in the comparison $ru-c^d$ is apparently not interfered with by the sex difference in viability of *ruru*, as witnessed by the insignificantly low value of $\chi_1^2 = 0{\cdot}52$. This is not the case for the comparison $e-ru$, where the segregation data, pooled for both sexes, have the significant χ_1^2 value of 5·73. Analysis of the data for each sex separately discloses that the lower viability of the male is the source of the disturbance ($\chi_1^2 = 5{\cdot}80$, table 4, line 12).

(iii) Combination of Data

For each of the contrasted pairs of mutants, estimates of the recombination fraction can be made more precise by suitable amalgamation of the data. For many of the gene pairs, data from both crosses of this paper may be used to augment that from the first paper. The combination is effected by summing the maximum likelihood scores (with due regard to sign) and the amount of information regarding p, yielded by each cross. The amount of information (I) is derived as the reciprocal of the variance for $p = 0{\cdot}5$. The score (S) and standard error (s) of p are also found for $p = 0{\cdot}5$.

The conclusions from the analysis of the combination data are shown by table 5. The total score and information will be useful for

TABLE 5

Summary of extent of linkage tests

Loci	p	s	S	I	Phase balance	Closest linkage
$e-ru$	0·477	0·018	−73·3333	3168·5913	75	0·465
$e-s$	0·487	0·028	−17.1111	1281·9118	61	0·445
$e-c^a$	0·522	0·033	20·8885	930·9622	0	0·436
$e-Y$	0·491	0·018	−28·0000	3254·2076	81	0·466
$ru-c^a$	0·473	0·033	−24·9835	938·1825	0	0·436
$ru-Y$	0·466	0·017	−113·3333 *	3331·9701	100	0·466
$s-c^a$	0·407	0·084	−12·8133	138·0122	100	0·335
$s-Y$	0·510	0·031	10·6667	1028·4231	■	0·439
c^a-Y	0·522	0·019	61·9999	2793·3332	□	0·463

* significant, see text

future amalgamations. Column six is an index of phase balance, conveniently tabulated as the percentage of the amount of information contributed by segregations in coupling linkage. Perfect balance is shown by the index 50, and possesses the implication that certain inviability interactions between genotypes which could pass undetected are, on the average, cancelled out. The last column gives the closest linkage compatible with the collated data and computed as $0{\cdot}5-1{\cdot}96s$. Table 6 arranges the χ^2 analysis for homogeneity.

Table 7 presents in detail the effect of the sex difference in viability of *ruru* animals upon the $ru-Y$ comparison. Consideration of the complete segregation would infer a genetic linkage ($p = 0{\cdot}4660 \pm 0{\cdot}0173$), if it were not for the inviability. Although the totals are significantly disturbed, this is probably a consequence of the reduced viability of *YXruru*, a crossover class. Consideration of the two non-ruby-eye classes supports the inviability suggestion, for in these the departure from random segregation is negligible ($p = 0{\cdot}5289 \pm 0{\cdot}0307$). This last estimate of p utilizes about 32 per cent. of the total information available for p but is compensated by the absence of bias.

The result of analyses of the three $ru-s$ segregations cannot distinguish between (*a*) an inviability interaction of genes *ru* and *s* in the

class *ruruss*, producing a larger than expectation of prenatal death of these animals, or (*b*) genetic linkage. For this reason, the *ru*—*s* pair are omitted from table 5. Crosses II and III show considerable dis-

TABLE 6

Homogeneity analysis of the segregations

Loci	Deviation from random assortment		Homogeneity		
	χ_1^2	*P*	χ^2	d.f.	*P*
e – *ru*	1·69	>0·10	7·22	4	>0·10
e – *s*	0·24	>0·50	4·94	2	>0·05
e – c^d	0·47	>0·30	1·14	1	>0·20
e – *Y*	0·24	>0·50	6·13	3	>0·10
ru – c^d	0·66	>0·30	0·25	1	>0·50
ru – *Y*	3·85 *	<0·05	6·60	4	>0·10
s – c^d	1·19	>0·20	...	...	...
s – *Y*	0·11	>0·70	3·46	2	>0·10
c^d – *Y*	1·38	>0·20	0·02	1	>0·95

* significant, see text

TABLE 7

Effect of differential viability of ru *upon linkage analysis of* ru-Y *segregation*

Cross	Segregation				Linkage analysis	
	RuY	*RuX*	*ruY*	*ruX*	Four classes χ_1^2	First two classes χ_1^2
I	170	195	26	33	0·02	1·71
II	110	113	55	69	0·83	0·04
III	180	197	53	45	1·33	0·77
IV	258	259	41	61	2·43	0
V	454	454	73	114	5·84 *	0
Total	*1172*	*1218*	*248*	*322*	3·85 *	0·89
Homogeneity (d.f. = 4)					6·60	1·63

* significant, see text

turbance from random assortment ($\chi_1^2 = 13{\cdot}34$ and 9·14, respectively), but cross IV does not ($\chi_1^2 = 0{\cdot}63$). The three crosses yield a total χ_3^2 of 23·12 ($P<0{\cdot}01$), which may be partitioned into deviation from random segregation of $\chi_1^2 = 17{\cdot}35$ ($P<0{\cdot}01$) and heterogeneity between crosses of $\chi_2^2 = 5{\cdot}77$ ($P>0{\cdot}05$). The heterogeneity χ_2^2 is large but is not significant. The weighted mean recombination fraction is $p = 0{\cdot}3183 \pm 0{\cdot}0342$ and is highly significant. At this junction it is not considered worthwhile to strive for a more accurate estimate of *p*. Attempts are in progress to improve the stamina of *ruruss* animals,

healthy individuals of which are necessary to produce an F_2 generation with *ru* and *s* segregating in opposite linkage phase (coupling) to the data so far obtained. The decision of linkage versus inviability interaction may be left open until suitably balanced data are procured.

4. DISCUSSION

The segregation data collected to date are sufficient to establish that the four mutant genes *e*, *ru*, *s* and c^a assort independently of each other and of sex. There is the possibility of linkage between *ru* and *s* but from other available evidence it is probable that the association is due to the enhanced inviability of one of the crossover classes (namely *ruruss*). The remoter possibility of linkage between *ru* and *Y* is also more probably due to a sex-differentiated inviability of a crossover class.

The question may be posed, what is the probability of detection of linkage in the hamster with four mutants? Green (1951) has proposed a formula for the computation of such probabilities given certain prior assumptions. The primary assumption is that the gene content of individual chromosomes do not differ too widely and, therefore, the probability of a mutant residing upon any chromosome is approximately the reciprocal of the haploid number. The Syrian hamster possesses 44 chromosomes (Husted, Hopkins and Moore, 1945; Matthey, 1952; Sachs, 1952; and Sheaffer, 1955), two of which are the sex determiners. It is probable that, except for weak sex-linkage, an association with sex would be noticed early in the study of a new mutant. Thus the probability may be found for the existence of at least one linkage among 4 genes for 21 autosomes. The probability is 26 per cent., hence it is not unduly surprising that no linkage has been uncovered. As a point of interest, the number of mutants necessary for the probability of linkage to exceed that of no-linkage is 6, but the probability is then merely 54 per cent. Green points out that unequal distribution of genes upon the chromosomes would in its effects appear to decrease the number of chromosomes. The above probability is perhaps, as a consequence, an underestimate. On the other hand, modal chiasma frequency per bivalent must be considered and this would tend to produce some very weak linkages at least, if not effective chromosome groups of pseudo-independent genes, depending upon the strength of localisation of the chiasmata. This would tend to reduce the above probability.

Since the formal genetics of the Syrian hamster has scarcely begun, it is urged that the collection of breeding data, which involves gene segregation amenable to linkage study, be published in full for future amalgamations (see Robinson, 1956). The possibility of weak sex-linkage should not be overlooked. This information may be compactly arranged in the manner of table 2 if pressure of other work prevents immediate analysis.

5. SUMMARY

1. A new mutant, partial albinism, is described, and the results of tests for linkage with other mutants and with sex are presented. No evidence for linkage could be found.

2. A critical collation of available segregation data for four mutant genes failed to disclose positive evidence for genetic linkage, either between the genes or with sex.

3. The joint segregation of two genes (ruby-eye, piebald) is disturbed but this could be due either to linkage or to an inviability interaction.

4. Since hamster genetics is in its infancy, a plea is made for the publication of all segregation data which could yield information upon weak linkage.

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AN ALGEBRAICALLY EXACT EXAMINATION OF JUNCTION FORMATION AND TRANSMISSION IN PARENT-OFFSPRING INBREEDING

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1. INTRODUCTION

THE Theory of Junctions in inbreeding was first sketched in the third chapter of the author's *Theory of Inbreeding* (1949). This was by no means a thorough treatment, being confined to the case of sib-mating. It was intended to illustrate the method by which the extent to which the germ-plasm is subjected to recombination in the course of a complete inbreeding programme, and thence the frequency with which at each stage the entire line becomes homogenic, can be calculated. In the author's opinion the course of events cannot be halted, or even greatly retarded, by moderate differences in viability; but, in the case of such bisexual organisms as the house-mouse must often be completed in forty or fifty generations.

In 1953 J. H. Bennett published in *Genetica* a paper on "Junctions in Inbreeding" giving comparative results for three other cases, namely (*a*) self-fertilisation in disomics, (*b*) self-fertilisation in tetrasomics, and (*c*) alternate parent-offspring mating in bisexual forms. In the last case it was remarkable that complete homogeneity appeared to set in some three generations earlier than in the case of sib-matings, which in many other respects it closely resembles.

The author has been struck by some minor discrepancies in the last series of results and, since the case is in some respects of especial simplicity, has been led to explore so far as to see if exact expectations at all stages could not be calculated instead of the asymptotic formulae he had previously used. Some inaccuracies in the original discussion have in the meanwhile been corrected in "A fuller theory of junctions in inbreeding" in *Heredity* (Fisher, 1954).

As will be seen in the following account, Bennett's conclusion that homogeneity is attained, at each level of probability, rather earlier by parent-offspring than by sib-matings is confirmed, but the difference appears to be less than was thought, ranging in the relevant region from about 1·8 to about 1·6 generations.

The symbols *a*, *b*, *c*, *d* are used to specify the tracts, in the neighbourhood of any particular point, introduced into the line by the foundation mating. The symbol *j* is used for a junction always taken to be between tracts *a* and *b*, so as to resemble *a* on one side and *b* on the other. With these conventions the various possible types of mating may be specified concisely. It seems probable that the small discrepancies mentioned above are due in part to inconsistencies in the numbering of the generations.

Without junctions, parent-offspring inbreeding starts with an unrelated mating, generation zero, leading to the first mating of offspring with parent. This is typically trigenic, but will lead to segregating digenic types of three kinds in accordance with the standard matrix.

Generation 0 $ab = cd$
" 1 $ac = ab$

Matrix

Offspring	ab	ab	ab	aa
Parent	ac	ab	aa	ab
Frequency	x	v	u'	u
$ab = ac$	2	–	–	–
$ab = ab$	1	1	1	–
$ab = aa$	–	–	–	1
$aa = ab$	1	1	1	–
	4	2	2	1

From this it appears, since in generation (1) the mating must be of trigenic type, that

$$x_n = (\tfrac{1}{2})^{n-1}$$
$$v_n = u_n = -\tfrac{1}{2}(\tfrac{1}{2})^{n-1} + \tfrac{1}{10}(1+6\epsilon)\epsilon^{n-1} + \text{conjugate}$$
$$u'_n = -\tfrac{1}{2}(\tfrac{1}{2})^{n-1} + \tfrac{1}{10}(2+2\epsilon)\epsilon^{n-1} + \quad " \quad .$$

Hence for $s > 0$, the fraction of the strands still heterogenic is

$$-(\tfrac{1}{2})^s + \tfrac{1}{10}(6+16\epsilon)\epsilon^s + \tfrac{1}{10}(14-16\epsilon)\,(\tfrac{1}{2}-\epsilon)^s$$

TABLE 1

s	*Numerator*	*Percentage*
0	1	100
1	2	100
2	4	100
3	7	87·5
4	12	75·0
5	20	62·5
6	33	51·5625
7	54	42·1875
8	88	34·375
9	143	27·92969
10	232	22·65625
11	376	18·35938
12	609	14·86816
13	986	12·03613
14	1596	9·72900
15	2583	7·88269

Table 1. Percentage of map length still heterogenic, illustrating the continuous method of calculation of the numerator

$$N_s = N_{s-1} + N_{s-2} + 1$$

to be divided by 2^s.

2. INTERNAL JUNCTIONS OF TYPE (a, b, j)

The trigenic and digenic types produce the internal junction type (δ), in particular,

$$aj = ab$$

with frequency $u'_s + 2v_s + \frac{1}{2}x_s$

or, appearing in generation $(s+1)$

$$-2(\tfrac{1}{2})^s + \tfrac{1}{10}(6+16\epsilon)\epsilon^s + \text{conjugate},$$

and of these the fraction $(\frac{1}{2})^{n-s-1}$ survive as internal junctions (a, b, j) to generation n. But

$${}_1S^{n-1}(\tfrac{1}{2})^{n-s-1}\{-2(\tfrac{1}{2})^s + \tfrac{1}{10}(6+16\epsilon)\epsilon^s + \tfrac{1}{10}(14-16\epsilon)\ (\tfrac{1}{2}-\epsilon)^s\}$$

is

$$\tfrac{1}{10}(14+44\epsilon)\epsilon^{n-1} + \tfrac{1}{10}(36-44\epsilon)\ (\tfrac{1}{2}-\epsilon)^{n-1} - (2n+3)\ (\tfrac{1}{2})^{n-1}.$$

Table 2 shows the calculation of these expected frequencies.

TABLE 2

Generation	N	(δ)	(α)
1	0	0	1·00000
2	2	·50000	·50000
3	8	1·00000	·25000
4	20	1·25000	·12500
5	42	1·31250	·06250
6	80	1·25000	·03125
7	144	1·12500	·01562
8	250	·97656	·00781
9	424	·82812	·00391
10	708	·69141	·00195
11	1170	·57129	·00098
12	1920	·46875	·00049
13	3136	·38281	·00024
14	5106	·31165	·00012
15	8296	·25317	·00006

Table 2. Expected numbers for 100 cM of map length of internal junction (a, b, j), with two original elements a, b, and a third derived from them by recombination. If either a or b is lost the junction is transmitted as an external junction.

Recurrence relation :—

$$N_s = N_{s-1} + N_{s-2} + (4s-6)$$

3. OTHER TYPES OF INTERNAL JUNCTIONS

The trigenic junctions (a, b, j) derived from

$$aj = ab$$

are the only kinds derivable from digenic matings, yet the foundation mating,

$$ab = cd,$$

and trigenic types,

$$ab = ac,$$

derived from it, yield three other kinds of internal junction.

(α) The type

$$cj = ab$$

involving both original strands a and b, the crossover strand j derived from them, and a strand c having nothing in common with the others, is completely transient, leading never to itself, but always to

$$ac = cj$$

or

$$aj = cj.$$

Being transient, the frequency is simply that of production by crossing-over in the previous generation. But, in the foundation mating, crossing-over occurring in the mate to be used a second time, leads to this type, while crossing-over in the other mate is ineffectual, since both the original strands are simultaneously lost.

Moreover, from the trigenic matings

$$ab = ac$$

it arises with frequency $\frac{1}{2}$ for each unit of map length, and therefore from generation 2 onwards, it must appear with frequency

$$2(\tfrac{1}{2})^n,$$

as also in the first generation.

These also are shown in table 2.

(β) The types (a, c, j) constitute the total output of (α), and therefore appear new in generation $n (n > 1)$ with frequency

$$4(\tfrac{1}{2})^n.$$

They are also half the output by recombination from the trigenic types and this supplies as much again by a different path.

These are $aj = ac$ and $cj = ac$.

The new production (for $s > 1$) is then

$$8(\tfrac{1}{2})^s,$$

and the type is maintained in half the descendants in each generation; consequently by generation n there has accrued

$$\sum_{s=2}^{n} 8(\tfrac{1}{2})^s(\tfrac{1}{2})^{n-s}$$

or

$$8(n-1)\,(\tfrac{1}{2})^n.$$

(γ) These types β, or (a, c, j) are of six kinds, with the generation matrix shown below

Offspring	Offspring Parent	*r* *ac* *cj*	*s* *aj* *cj*	*t* *aj* *ac*	*u* *cj* *ac*	*v* *ac* *aj*	*w* *cj* *aj*
ac	*cj*	.	.	.	1	.	1
aj	*cj*	.	.	.	1	.	1
aj	*ac*	1	.	.	.	1	.
cj	*ac*	1	.	.	.	1	.
ac	*aj*	.	1	1	.	.	.
cj	*aj*	.	1	1	.	.	.
cj	*cj*	.	.	.	1	.	1
ac	*cj*	.	.	.	1	.	.
jj	*cj*	.	.	.	.	.	1
		4	4	4	4	4	4

Latent Root	Principal Component
1	$r-s$
0	$t-u$
0	$v-w$
$-\frac{1}{2}$	$2r-s-t-u+2v-w$
$-\frac{1}{2}$	$r-u+v-w$
$\frac{1}{4}$	$r+s+t+u+v+w$

The first four are supplied anew at rate $2(\frac{1}{2})^s$ each, when $s>1$; after $n-s$ generations

$$u_n+w_n=\tfrac{1}{3}\{4(\tfrac{1}{2})^{n-s}-(-\tfrac{1}{4})^{n-s}\}(\tfrac{1}{2})^{s-1},$$

summed from $s=2$ to n, this is

$$\tfrac{4}{3}(n-\tfrac{7}{6})\ (\tfrac{1}{2})^{n-1}+\tfrac{2}{9}(-\tfrac{1}{4})^{n-1},$$

so that a new production of type (γ) appears in the n^{th} generation with frequency

$$\tfrac{4}{3}(n-\tfrac{13}{6})\ (\tfrac{1}{2})^{n-1}-\tfrac{4}{9}(-\tfrac{1}{4})^{n-1}$$

from $n=3$.

For the last enumeration then we may note that the probability of these remaining after $s-n$ further generations, as a junction (c, j) of type (γ), is

$$\tfrac{1}{10}(3+8\epsilon)\epsilon^{s-n}+\tfrac{1}{10}(7-8\epsilon)\ (\tfrac{1}{2}-\epsilon)^{s-n}.$$

The process of summation from $n=3$ to $n=s$ is somewhat intricate, but the result is similar to those of the other cases. Algebraically, it is

$$-(4n+2)2^{-n}+\tfrac{1}{5}(6+16\epsilon)\epsilon^n+\tfrac{1}{5}(14-16\epsilon)\ (\tfrac{1}{2}-\epsilon)^n,$$

which can be evaluated continuously by the recurrence formula

$$N_s=N_{s-1}+N_{s-2}+(4s-10)$$

dividing at each stage by 2^s.

Table 4 summarises the expected numbers per 100 cM of strand, of the four types of internal junctions.

TABLE 4

	α $cj = ab$	β (a, c, j)	γ (c, j)	δ (a, b, j)	Total internal junctions
1	1·00000	—	—	—	1·00000
2	·50000	2·00000	—	·50000	3·00000
3	·25000	2·00000	·25000	1·00000	3·50000
4	·12500	1·50000	·50000	1·25000	3·37500
5	·06250	1·00000	·62500	1·31250	3·00000
6	·03125	·62500	·65625	1·25000	2·56250
7	·01562	·37500	·62500	1·12500	2·14062
8	·00781	·21875	·56250	·97656	1·76562
9	·00391	·12500	·48828	·82812	1·44531
10	·00195	·07031	·41406	·69141	1·17773
11	·00098	·03906	·34570	·57129	·95703
12	·00049	·02148	·28564	·46875	·77637
13	·00024	·01172	·23438	·38281	·62915
14	·00012	·00635	·19141	·31165	·50952
15	·00006	·00342	·15582	·25317	·41248
Asymptotic	$2{\cdot}2^{-n}$	$8(n-1)2^{-n}$	$-(4n+2)2^{-n}$ $\frac{1}{5}(6+16\epsilon)\epsilon^n$	$-(4n+6)2^{-n}$ $\frac{1}{5}(8+28\epsilon)\epsilon^n$	$-14{\cdot}2^{-n}$ $\frac{1}{5}(14+44\epsilon)\epsilon^n$

TABLE 5

	Total internal	Total junctions	External
1	1·00000	1·00000	—
2	3·00000	3·00000	—
3	3·50000	4·50000	1·00000
4	3·37500	5·25000	1·87500
5	3·00000	5·56250	2·56250
6	2·56250	5·56250	3·00000
7	2·14062	5·35938	3·11875
8	1·76562	5·03125	3·26562
9	1·44531	4·63281	3·18750
10	1·17773	4·20312	3·02539
11	·95703	3·76855	2·81152
12	·77637	3·34668	2·57031
13	·62915	2·94849	2·31934
14	·50952	2·58032	2·07080
15	·41248	2·24594	1·83276
	$-14{\cdot}2^{-n}$ $+\frac{1}{5}(14+44\epsilon)\epsilon^n$	$\frac{1}{5}(-2+8\epsilon)\epsilon^n$ $+\frac{n-1}{5}(6+16\epsilon)\epsilon^n$	$14{\cdot}2^{-n}$ $+\frac{1}{5}(6+16\epsilon).$ $(n-5+2\epsilon)\epsilon^n$

The total of junctions current in generation n is

$$\tfrac{1}{5}(-2+8\epsilon)\epsilon^n+\tfrac{1}{5}(2-8\epsilon)\ (\tfrac{1}{2}-\epsilon)^n$$

$$+\frac{n-1}{5}\{(6+16\epsilon)\epsilon^n+(14-16\epsilon)\ (\tfrac{1}{2}-\epsilon)^n\}.$$

If from this is subtracted the total of internal junctions, the difference representing at each stage the expected number of external junctions, is given by the general formula

$$\begin{array}{r} 14{\cdot}2^{-n} \\ +\frac{1}{5}(n-1)\ (6+16\epsilon)\epsilon^n+\text{conjugate} \\ +\frac{1}{5}(16+36\epsilon)\epsilon^n \qquad + \qquad ,, \end{array}$$

but

$$16+36\epsilon=(6+16\epsilon)\ (4-2\epsilon)$$

so the expected number of external junctions may be written

$$\tfrac{1}{5}(n-5+2\epsilon)\ (6+16\epsilon)\epsilon^n \cdot 14\cdot 2^{-n} + \text{conjugate term.}$$

TABLE 6

	m	Probability e^{-m}
25	5·30832	·00495
26	4·48609	·01126
27	3·78430	·02272
28	3·18695	·04130
29	2·67973	·06858
30	2·25001	·10540
31	1·88669	·15157
32	1·58007	·20596
33	1·32176	·26667
34	1.10448	·33139
35	·92198	·39773
36	·76891	·46352
37	·64068	·52693
38	·53338	·58662
39	·44369	·64167
40	·36881	·69155
41	·30635	·73613
42	·25429	·77547
43	·21095	·80982
44	·17488	·83955
45	·14490	·86511
46	·11999	·88693
47	·09931	·90546
48	·08215	·92114
49	·06792	·93434
50	·05614	·94541
51	·04637	·95468
52	·03829	·96244
53	·03160	·96889
54	·02608	·97375
55	·02151	·97872
56	·01773	·98242
57	·01461	·98549
58	·01204	·98803
59	·00992	·99013
60	·00816	·99187

Note.—The distribution of the probability of elimination of the last heterogenic tract by generations would be very similar to that shown in Fig. 1 of Fisher (1954) for sib-mating, but about $1\frac{1}{4}$ generations earlier.

4. THE NUMBERS OF HETEROGENIC TRACTS

For a number of generations exceeding 20, only the leading terms need be considered. If L is the total length of strand, the number of external junctions expected will be

$$\tfrac{1}{5}(n-5+2\epsilon)\ (6+16\epsilon)\epsilon^n L$$

and if ν is the number of chromosomes, the number of chromosome ends still heterogenic will be

$$\tfrac{1}{5}(6+16\epsilon)\epsilon^n\ \nu.$$

The expected number of heterogenic tracts will be half the sum of these, or

$$m = \tfrac{1}{8}(3+8\epsilon)\epsilon^n\{(n-5+2\epsilon)L+\nu\}.$$

Putting, as is approximately appropriate for mice,

$$L = 25,\ \nu = 20,$$

it appears that at 25 generations m has fallen to about 5·3, and the probability that the whole material is homogenic, e^{-m}, is just under 1 per cent. The value up to $n = 60$ of m and of the derived probability, are shown in table 6.

The values shown in table 6 correspond closely to the corresponding probabilities for sib-matings published in 1954. The first quartile, 25 per cent., is passed between the 32nd and the 33rd generations, whereas with sib-mating it falls between the 34th and the 35th. Fifty per cent. is passed between generations 36 and 37 instead of between 38 and 39, while the third quartile, 75 per cent., is between generations 41 and 42, instead of 43 and 44. These comparisons suggest a difference of two generations, while a more accurate interpolation of the probabilities gives a difference of about 1·8 at 25 generations falling very slightly to about 1·6 at 60 generations, when in both cases complete homozygosis is almost certainly attained.

5. SUMMARY

For the comparatively simple system of inbreeding by alternate parent-offspring matings exact algebraic expressions can be found for the proportion of the germinal strands expected at each stage to be heterogenic, and for the expected number of junctions, external and internal, at each stage. The internal junctions are of four kinds, three of which are derived in a kind of cascade, requiring fairly careful analysis. It seemed therefore useful to explore the possibility of such an exact examination.

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EFFECTS OF DISRUPTIVE SELECTION

I. GENETIC FLEXIBILITY

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THIS article, and those which will follow it, concern the results of experiments using artificial selection of the type that Mather (1953*a*) has called *disruptive* and Simpson (1944) has called *centrifugal*. Such selection may be said to occur when we maintain a single population by choosing more than one class of individuals to provide the parents of each generation. In its extreme form disruptive selection involves choosing both extreme classes and discarding intermediates.

The antithesis of disruptive selection is *stabilising selection* (Simpson's centripetal selection), which occurs when we choose, as parents of each generation, individuals at, or as close as possible to, the mean of the population from which they come.

Mather's third type of selection is the more usual *directional selection* whose effects have been widely studied.

Cyclic selection (Thoday, 1956) provides a fourth type which would occur if we were to reverse the direction of selection in different generations. It clearly has something in common with disruptive selection as Mather (1955*a*) has pointed out.

Waddington (1953, 1958) has classified selection according to the effects it may be expected to produce, rather than according to the measurable characters of the individuals selected. While of value for theoretical discussion, such a classification cannot be used in designing selection experiments and is not therefore used here.

Schmalhausen (1949), Mather (1953*a*, 1955*a*, *b*) and Waddington (1953, 1958) have discussed the consequences that might be expected to result from different types of selection. In principle we must expect effects of two kinds. Responses might occur as changes in the effective variety of genotypes, or as changes in the variability of development. The first would involve change in the amount of effective genetic variation in the population. The second would involve change in the responsiveness of the developmental system to the environmental variance to which the population is exposed, or changes in the amount or effectiveness of the accidental sources of developmental variance that Waddington, Graber and Woolf (1957) call developmental noise. We may therefore expect stabilising selection to reduce the genetic variation in a population (Waddington's normalising selection) or to increase the stability of the developmental processes mediated by the genotypes in the population (Waddington's stabilising or canalising selection), or to do both. Disruptive selection might be

expected to have the opposite effects, increasing genetic variation and/or increasing developmental flexibility by producing epigenetic systems with alternative pathways. It might also act to reduce developmental stability or the canalisation of existing pathways.

Mather (1955*a*) has further argued that disruptive selection should have more profound effects. Provided that the two or more types selected are necessary to one another, disruptive selection might be expected to give rise to a polymorphic situation based on alternative pathways of development. Development might be switched into one or other path by a genetic switch mechanism, or by an environmental switch which, if available, should be equally effective and might equally well be exploited. On the other hand, if the two or more types selected are not dependent on one another, then disruptive selection could put a premium on the development of an isolation barrier and lead to the separation of different populations with different characteristics.

Mather's predictions imply that disruptive selection may be of the greatest evolutionary significance. Yet few experiments have been carried out to determine how effective either disruptive selection or stabilising selection may be. Falconer and Robertson (1956) compared the effects of stabilising and disruptive selection for weight in mice and Falconer (1957) has studied the effect of stabilising selection on abdominal chaeta-number in *Drosophila*. Neither of these experiments gave marked results though there was some reduction in variance in the mouse stabilising line. The present paper describes the results of similar experiments using sternopleural chaeta-number in *Drosophila melanogaster*. A preliminary account has already been published (Thoday, 1958*a*).

1. MATERIAL AND CULTURE METHODS

All the experiments have been carried out in lines that originate from a single wild stock " Dronfield." This wild stock derived from a single fertilised female captured near Sheffield in May 1954, and has been maintained at 25° C. (approximately) ever since, usually by 4-pair transfers. It is the same stock as was used for the directional selection experiments described by Thoday (1958*b*).

Details of culture are similar to those previously described (Thoday, 1958*b*). Each line is maintained by 4 cultures in each generation (three weeks per generation), each culture having a single pair of parents. The 4 cultures are labelled according to the origin of their mothers and represent 4 separate female sub-lines. A culture is assayed by counting chaeta-numbers on both sides of 20 flies of each sex, and the best 8 of each sex are selected. Of any such 8 the best is intended to continue the line, but the second, third and fourth are set up as insurance cultures in case the first fails. (When the best successful culture seemed likely to produce very few flies, virgins were sometimes collected from a second culture and used to provide extra flies to complete the assay.) The remaining 4 are set up together in a fifth (" mass ") culture to ensure absolutely against the loss of a female line. (It has only been necessary to use 8 of these in the experiments described here.) Thus each line is

set up as 16 single-pair cultures and 4 four-pair cultures, though the aim is only to use 4 single-pair cultures, one from each female line. This procedure is designed to ensure against loss of female lines, and is necessitated by the insistence on single-pair cultures.

If, in selection, choice had to be made between two flies of equal chaeta-number, the more bilaterally symmetrical was chosen.

2. MATING AND SELECTION SYSTEMS

(i) Disruptive selection with negative assortative mating: the D⁻ line

The first disruptive selection line to be established was primarily intended to assess the possibility that selection might be able to bring

TABLE 1

The mating and selection systems

	Parents of Generation	Culture (*i.e.* female sub-line)							
		A		B		C		D	
		♀	♂	♀	♂	♀	♂	♀	♂
(*a*)	D⁻								
	n	HA × LC		HB × LD		LC × HA		LD × HB	
	n+1	HA × LD		HB × LC		LC × HB		LD × HA	
	n+2	HA × LC		HB × LD		LC × HA		LD × HB	
	n+3	HA × LD		HB × LC		LC × HB		LD × HA	
	etc.								
(*b*)	D⁺								
	n	HA × HC		HB × HD		LC × LA		LD × LB	
	n+1	LA × LD		LB × LC		HC × HB		HD × HA	
	n+2	HA × HC		HB × HD		LC × LA		LD × LB	
	n+3	LA × LD		LB × LC		HC × HB		HD × HA	
	etc.								

The entries designate the parents used to produce the culture in the generation shown in the first column. H indicates the highest, and L the lowest chaeta-number fly found in the appropriate culture. A, B, C and D indicate the culture from which the fly was selected.

about responses in the cytoplasm, and a preliminary report has been given (Thoday, 1958*c*) of the results from this point of view. The mating and selection system is given in table 1*a*.

(ii) Disruptive selection with positive assortative mating: the D⁺ line

This line was set up specifically to test the effects of disruptive selection, and was designed to ensure that cytoplasmic variables, if any, would not be subjected to consistent selection. The mating and selection system used is given in table 1*b*. This system ensures that there is selection for high and for low chaeta-number flies in each generation, but that high and high will be mated together, and low and low will be mated together in separate cultures.

At generation 21 of this line an unfortunate error was made in selection. *For this one generation the whole line was selected for low chaeta-number.* This must be borne in mind when the results are considered.

(iii) Stabilising selection: the S line

This line is maintained by exactly the same mating system as shown in table 1*b*, and originated from the generation 1 cultures of the D^+ line. It was intended to provide a comparative line. The flies selected in each generation are those with chaeta-numbers nearest to the mean of the wild stock from which the lines originated. This mean has varied from 17 to 18 chaetæ, and, as males usually have rather fewer chaetæ than females, the aim is always to select females with 9 chaetæ on each side, and males with 9 on one side and 8 on the other. This aim has usually but, of course, not always been achieved. This mean of 17·5 proved a little low, so that there has been slight directional selection as well as stabilising selection.

(iv) Divergent-directional selection

Certain divergent-directional selection experiments have been carried out on the lines to test their responsiveness to directional selection. Each of these involved taking coincidentally a high selection line and a low selection line and observing their divergence over three generations. Each of these lines was maintained with 4 single-pair cultures per generation, a rotational mating system being used exactly as described in Thoday (1958*b*). The four initial cultures always included all four female sub-lines of the line under test. These test lines were run at a generation every two weeks (not three weeks as for the main lines) for three generations.

3. CHAETA-NUMBER, ASYMMETRY AND VARIANCE IN THE LINES

Fig. 1 shows the mean chaeta-numbers, arithmetic asymmetries and within-culture-and-sex mean squares for the three lines. The asymmetry values are means of the differences between the sides of the flies, sign ignored. No correction for relation between asymmetry and mean (Thoday, 1955, 1958*b*) has been made. Neither are the variances corrected for any comparable relation to mean.

The generation numbers in the figure are those applicable to the D^- line. Coincident generations for the three lines as plotted were cultured coincidentally.

(i) The D⁻ line

The selection practised on the D^- line clearly had negligible effect on mean chaeta-number in the first 10 generations. Neither variance nor asymmetry show evidence of a trend during this period.

From generation 10 to generation 17, the mean rose slowly but

steadily and this was accompanied by wide fluctuations of variance which, however, was higher after this period. At the same time asymmetry rose sharply to a new level, a rise that coincided with and appears to have been confined to the period of most rapid response

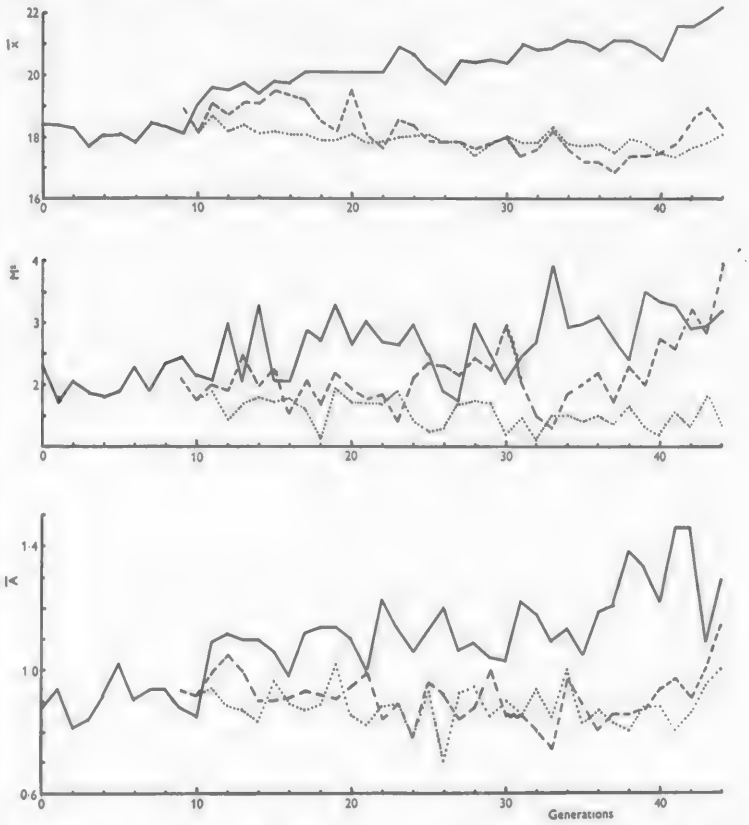

FIG. 1.—Mean chaeta-numbers ($\bar{x}$), within-sex-and-culture mean squares (M^2) and asymmetries ($\bar{A}$) in the three lines. Solid line D^-: broken line D^+: dotted S. The generations are those applicable to D^-: points plotted together represent data obtained from coincidentally raised cultures. The generation at which the selection error was made in D^+ is marked by a wavy line.

in mean. It is clear that events during this period were complex. Variance and asymmetry can both be correlated with mean and part of their rise is likely to be due to this correlation. But the fluctuations of variance, and the suddenness of the rise in asymmetry suggest that other factors are involved.

From generation 17 to generation 22 the mean was very steady

indeed and the variance, though fluctuating, showed no overall change. Thereafter the mean fluctuated a little but rose slowly, variance fluctuated widely but showed a further overall rise, and asymmetry behaved similarly though its rise seems more striking in recent generations.

Apart from the rise of mean chaeta-number there is little clear evidence of any change in this line that could be attributed to disruptive selection, and the results are essentially the same as those of the corresponding mouse line of Falconer and Robertson (1956). The overall rises in variance and in asymmetry may be attributable to correlations of these measures with mean. However, inspection of the curves does suggest that changes are occurring. The relative steadiness of variance at the beginning of the experiment suggests that the fluctuations that occurred later have some real meaning, and the correlation of asymmetry with mean seems far from complete. There is also a suggestion of a cyclic behaviour of asymmetry which is subject to rises and falls, as if selection were occasionally picking out developmentally unstable genotypes, but that these were then being eliminated by natural selection. It seems that both variance and asymmetry are subject to complex causes of changes. Some of these causes may counteract one another, and some will be independent of the artificial selection. The comparatively negative results for variance cannot therefore be critical evidence that variance is little affected by the artificial selection.

(ii) The D+ line

The mean chaeta-number of this line rose during the first 9 generations and then fell until it coincided with that of the S line. Variance behaved likewise at first. There were two fluctuations of mean, the more notable being that at generation 11 (Generation D^- 20 in fig. 1). Since this coincides with a period of very stable mean in D^-, and is not reflected in S, it seems at first rather unlikely that some environmental fluctuation can have been responsible. However, the single generation rise in mean occurred in all four cultures of the line and an environmental factor to which only the D^+ line was responsive seems to be the most probable cause.

After these fluctuations the D^+ mean remained virtually identical with that of S until, at generation 21 (D^- 30), the error of selection was made and it fell 0·5 chaetæ. It then remained below that of S until the most recent generations. During the period of stable mean, variance rose until at generation 21 (D^- 30) it had reached the level characteristic of line D^-. Over this period the variance of D^+ was clearly greater than that of S, though their means were the same. There is no suggestion that their asymmetries were different, and it therefore seems very likely that disruptive selection during this period caused an increase in the effective genetic variation in the D^+ line, though of course asymmetry can only be a very partial measure

of non-genetic variance. The error in selection made in generation 21 (D^+ 30) resulted in the loss of this increased within-sex-and-culture variance. It has, however, been regained since. Variance has now risen above that of D^- and is still rising.

TABLE 2

Coefficients of within-sex-and-culture variation (per cent.) and coefficients of asymmetry ($A/T \times 1000$)

D^- Generations		0	1	2	3	4	5	E	7	E	9	10	11	12	13	14
D^-	CV	8·4	7·1	8·4	7·7	7·4	7·6	8·4	7·5	8·4	8·7	7·7	7·5	8·9	7·2	9·4
	A/T	48	51	45	47	51	56	50	51	51	49	45	55	58	55	57
D^+	CV										7·5	7·3	7·5	7·3	8·2	7·2
	A/T										49	51	52	56	53	48
S	CV											7·3	7·5	6·5	7·1	7·5
	A/T											51	51	49	48	47

D^- Generations		15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
D^-	CV	7·1	7·3	8·4	8·2	9·0	7·3	8·9	8·3	7·8	8·2	7·9	6·9	6·4	8·5	7·7
	A/T	53	50	55	57	57	55	50	62	55	51	56	61	52	53	51
D^+	CV	7·6	6·3	7·6	6·8	8·2	7·0	7·3	7·8	6·3	7·9	8·6	8·6	8·1	8·9	8·4
	A/T	45	48	48	50	50	49	55	47	48	42	54	51	46	50	56
S	CV	7·1	7·5	7·1	5·9	7·9	7·2	7·4	7·2	7·7	6·7	6·2	6·4	7·3	7·4	7·3
	A/T	45	50	49	51	58	48	46	50	49	43	52	40	52	55	47

D^- Generations		30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
D^-	CV	7·0	7·5	7·9	9·6	8·0	8·1	8·5	7·9	7·3	8·8	8·4	8·4	7·7	7·8	8·0
	A/T	50	58	57	52	53	50	57	61	65	63	58	66	67	48	58
D^+	CV	9·6	8·3	6·9	6·1	7·8	8·2	8·6	7·5	8·8	8·1	9·5	9·1	9·6	8·8	10·9
	A/T	47	49	41	40	55	52	47	51	49	50	54	54	49	54	62
S	CV	6·6	6·8	5·9	6·6	6·8	6·6	6·9	6·7	7·2	6·4	6·3	7·3	6·5	7·7	6·3
	A/T	51	47	45	46	56	47	49	48	45	49	50	46	49	53	55

Note.—This table and figure 1 have been completed to the time of going to press. Other tables and computations do not include the most recent generations.

(iii) The S line

The mean of this line, initially a little over 18 chaetæ, declined slowly, as is to be expected since the parents in each generation averaged 17·5 chaetæ. Variance and asymmetry suggest little, if any, significant change though variance declined a little.

4. COEFFICIENTS OF VARIATION

The differences of mean chaeta number distinguishing D^- and the other lines, and at times distinguishing D^+ and S, make it difficult to interpret comparisons of the variances of these lines. Coefficients of variation are often used in such situations, though coefficients of variation involve assumptions about the relation between variance and mean which are difficult to justify. Despite the problems to which these assumptions give rise, it seems worth comparing the lines in this way and table 2 lists the coefficients. There is no evidence of change

of the coefficient of variation of D⁻. On the other hand there is evidence of a rise with generations in D⁺ and a fall in S. The regression of coefficient of variation on generations for D⁺ is positive and just significant ($t_{(28)} = 2{\cdot}03$, $P \simeq 0{\cdot}05$) and that for S is negative and significant ($t_{(28)} = 2{\cdot}48$, $P \simeq 0{\cdot}02$). The two regression lines meet at generation 0. Table 3 gives the results of a joint analysis of variance for these two lines, showing that the joint regression is not significant, the two regressions are significantly different, and D⁺ has a significantly higher mean than S. D⁺ disruptive selection has raised, and stabilising selection has lowered the coefficient of variation.

If we accept coefficients of variation as meaningful, we must conclude that D⁺ disruptive selection has raised and stabilising selection has lowered variance. D⁻ selection has failed to raise coefficient of

TABLE 3

Analysis of variance of coefficients of variation for the D⁺ and S lines

Source	*n*	Mean Square	P
Joint Regression on Generations.	1	16·1580	≃0·5
Difference between Regressions .	1	355·4631	<0·01
Difference between Means. .	1	968·0166	Small
Error	56	42·1597	...

variation, though it has raised mean and uncorrected variance. It is of course quite likely that the relation of variance to mean differs in different lines, and that the negative result for D⁻ is not real.

5. COEFFICIENTS OF ASYMMETRY

Sternopleural asymmetry is a partial measure of the stability of development (Thoday, 1958*b*), but, like variance, it may also be related to mean (Mather, 1953*b*) so that comparison of the asymmetry of lines whose means are different is problematical. The base stock, " Dronfield," used for these experiments is the same as that used for those described in Thoday (1958*b*), in which what may be called a coefficient of asymmetry was used to correct for the scaling problem. This was calculated by dividing mean asymmetry (A) by mean chaeta-number (T). Those experiments provided evidence that the correction was satisfactory for this stock and it therefore seems justifiable to use it here. Table 2 lists the A/T values (multiplied by 1,000 for convenience). There is clearly no evidence of change of A/T in D⁺ or S or of difference between them in this respect. A/T has, however, risen in D⁻. Its initial value (×1,000) is of the order of 50 and is the same as that for D⁺ and S and for the Dronfield stock when the experiments described in Thoday (1958*b*) were begun. This seems a stable and characteristic value of A/T in this stock in our culture conditions.

The value has, however, risen in the D⁻ line. The regression on generations is positive and significant ($t_{(40)} = 4{\cdot}7$, P small). It seems likely, however, that the rise has not been consistent, but that it is entirely attributable to two periods in the history of the line, one at about generation 11 and the other at about generation 36. Table 4 gives the analysis of variance of D⁻ A/T for generations 0-41, the generations being combined into seven blocks of 6 generations each. Two of the six degrees of freedom for blocks of generations absorb all the significant variance. These two degrees of freedom correspond to the divisions between generations 11 and 12 and between generations 35 and 36. The first rise is that evident in fig. 1 and coincides with the first sharp rise of mean chaeta-number. Here selection of higher chaeta-number genes may have caused a deterioration of developmental stability as it did in the lines described in Thoday (1958*b*).

TABLE 4

Analysis of variance of A/T for the D⁻ line, generations 0-41
(Six-generation blocks)

Source	n	Mean Square	P
Blocks 1 + 2 v. rest . . .	1	310·2880	<0·001
Block 7 v. rest	1	246·5334	<0·001
Residual blocks . . .	4	6·0208	...
7 six-generation blocks . .	6	96·8175	<0·001
Within blocks (Error) . .	35	12·8048	...

The second rise, at generation 36, occurred in a period of stable mean and presumably reflects a direct response of developmental stability to disruptive selection.

The indications would seem to be that disruptive selection can pick out genotypes that decrease developmental stability, but that the resulting effect is slight and is rarely permanent. Of the rises in asymmetry and A/T that occurred in D⁻, only the two discussed above were sustained. Others occurred, notably at generations 17, 22 and 31, but each was followed by a fall as if natural selection were subsequently eliminating them. It must be concluded that stability of development as measured can respond but responded little to the types of selection used here. There is certainly no evidence of steady deterioration in both the D lines or of improvement in S.

It may seem that the rises in A/T which did occur in the D⁻ line should be reflected in the variances or coefficients of variation. The correlation between coefficient of variation and A/T for the D⁻ line is positive but very insignificant, so that there is no evidence of such reflection. Correlations between asymmetry and variance can occur (Thoday, 1955; Beardmore, unpub.) and might be expected to be evident in these data. That they cannot be detected may indicate that other more important causes of variation of variance are operating.

6. FERTILITY

The data available provide two measures of the fertility of the lines. Records have been kept of the cultures that failed in each generation, and of the number of flies collected in the culture used to assay each female line (except on the 8 occasions when all 4 single-pair cultures of a female sub-line failed and the mass cultures had to be used).

The failure rates are (per culture) 0·187 for D^-, 0·184 for D^+ and 0·254 for S. There is no evidence of difference between coincident generations of D^- and D^+ in this respect, and there is no evidence of a consistent trend of failure rate in D^- as the experiment progressed. The difference between S and D^+, however, is significant (100 failures

TABLE 5

The number of single-pair cultures that failed.
(*From 16 cultures set up per line per generation*)

D^- Generations	D^-	D^+	S
0-10	18	...	...
11-21	46	40	48
22-32	42	37	43
33-43	19	23	43

out of 528 cultures in D^+, 134 out of 528 in S, $\chi^2_{(1)} = 6{\cdot}3465$ $P<0{\cdot}02$) and there is evidence suggesting that this difference has increased with generations. (Comparing the lines in the first 11 generations $\chi^2_{(1)} = 0{\cdot}9697$, $P>0{\cdot}3$, the second 11 generations $\chi^2_{(1)} = 0{\cdot}5823$, $P>0{\cdot}3$ and in the third 11 generations $\chi^2_{(1)} = 7{\cdot}4592$, $P<0{\cdot}01$, though the lines × generations χ^2 is not quite significant.) If this increase of difference between the lines is real it is a little difficult to interpret, for it occurs, not as increase in the number of failures in S, but as decrease of failures in D^+ (table 5). The only firm conclusion we can draw, therefore, is that failures are more frequent in the S line than the others. However, the D^- data would suggest that culture conditions or the handling of the flies has varied, thus masking deterioration of fertility in S and giving a spurious improvement in D^+. That S has in fact deteriorated is clear from other evidence (see below).

The data for productivity are more informative, despite the large error such data have. The total flies recorded for D^+ and S (the two strictly comparable lines) are summarised in table 6. Table 7 presents the results of an analysis of variance of the D^+ and S productivities. It seems clear that productivity has declined significantly in both lines, that it has declined more in S than D^+, and that S is very much less productive than D^+. D^- figures are similar to those for D^+. That fertility declines with selection is well known (Mather and Harrison, 1949), but it seems surprising that stabilising selection should be the

more effective in producing such a decline in the present experiments. Quite apart from these data, handling the lines themselves gives a strong impression that the S line has become very poor indeed. Its

TABLE 6

Mean numbers of flies collected per culture in D+ and S

Generations	D−	S
First 8	154	134
Second 8	128	91
Third 8	103	76
Fourth 8	122	77
Total	*127*	*95*

lack of vigour is similar to that of a poor inbred line or a newly-plateaued selection line and suggests that stabilising selection in conjunction with small population size may have led to increasing homozygosity and consequent unbalance.

TABLE 7

Analysis of variance of productivities of cultures: D+ and S

Source	*n*	Mean Square	P
Lines	1	63409	Small
Generation Blocks	3	39833	Small
Lines × Generation Blocks	3	2758	<0·001
Residual Generations	28	6011	Small
Cultures, etc. (Error)	219	389	...

7. RESPONSIVENESS TO DIRECTIONAL SELECTION

It is clear that, though it provides positive clues in the D+ line and perhaps in S, variance cannot be relied upon in a negative sense as an indicator of the effects of disruptive or stabilising selection. There are too many factors that may cause it to change and some of them at least (*e.g.* inbreeding in the S line) may act to change variance in directions opposite to those in which the artificial selection might be expected to change it.

Such difficulties were anticipated when the experiments were initiated and it was planned to test the lines, using divergent directional selection experiments, to determine how much free genetic variation they possessed. Such tests have been carried out on D− at generations 21, 27 and 32; on D+ at generations 13 and 23 and 34 (= D− 22, 32 and 43); and on S at generations 11, 12, 17 and 22 (= D− 21, 22, 27 and 32).

The results are presented in fig. 2 in which the rates of divergence of High and Low line means are plotted. Results are also given in

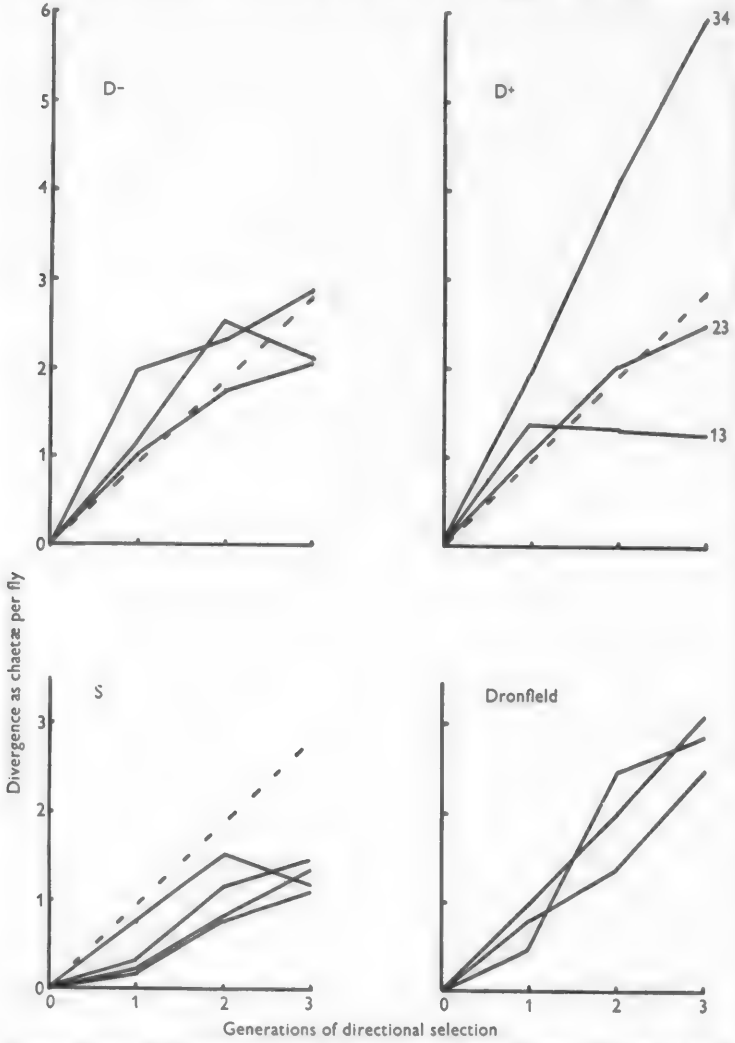

FIG. 2.—Results of divergent-directional selection tests on the lines and on the base stock (Dronfield) from which they were derived. Each solid curve represents the difference, in chaetæ per fly, between a high and a low selection line. The broken line represents the mean result of the three tests on the base stock. The generations in which D^+ was tested are indicated and are those plotted with D^- 22, 32 and 43 in Figure 1.

this figure for comparable tests of the wild Dronfield stock from which all the lines derive. Two of these are taken from the data of Thoday

(1958*b*), and the third was carried out two and a half years later (coincidentally with the tests on generation 27 D⁻ and 17 S). The agreement between these three testifies to the stability of this stock.

It is clear from the results of these tests that the D⁻ line and the S line differ. They were in fact already different when first tested and there is no evidence of subsequent further change. The D⁻ line is responsive to selection, and the S line is much less so. The S line is less responsive to selection than the Dronfield stock from which it came. The D⁻ line seems to respond more than the Dronfield stock to one generation of selection, but thereafter its response decreases so that after three generations of directional selection it has diverged no more and perhaps less than the stock.

Two of the D⁺ line tests were made at unfortunate times. The first test at generation 13 was made before variance had begun to rise in the line. It gave results in the first generation of directional selection comparable with those of the D⁻ line, but there was no further response. It was not possible to arrange to test it again until shortly *after* the error in selection had been made. It then (generation D⁺ 23 = D⁻ 32) responded exactly as the base stock. Bearing in mind that the selection error in D⁺ 21 (= D⁻ 30) had lowered the mean-square of this line from about 2·5 to about 1·5, this response is remarkable. D⁺ was tested again at generation 34 (= D⁻ 43) after it had regained a higher variance. At this test it proved most responsive, and there seems no doubt that it is now much more responsive than the base stock from which it was derived.

Together these tests make it quite clear that the D lines contain more effective genetic variation than the S line. They are genetically more flexible. There is also a suggestion that the genetic variation in the D⁻ line is more readily exploited by one generation of selection than is that in the Dronfield wild stock, and there is no doubt that disruptive selection has made the D⁺ line considerably more responsive than the stock.

These results may be used to provide estimates of heritability (realised heritability) from the formula $h^2 = \frac{d\mathrm{O}}{d\mathrm{P}}$ (where dO is the difference between the high and low line means after 1 generation of selection and dP is the difference between the selected parents used to produce the two lines). The formula is equivalent to $h^2 = \Delta\mathrm{P}/_i$ (Lerner 1950).

The combined results for the first generations of all the divergent directional selection tests give $h^2 = 0{\cdot}29$ for D⁻, 0·26 for D⁺, 0·15 for the base stock, and 0·09 for S. Heritability estimates obtained from the progeny tests of D⁻ and S figured in Thoday (1958*a*, fig. 3) are 0·20 for D⁻ and 0·05 for S, and are in reasonable agreement with those above.

8. DISCUSSION

Natural populations of outbreeding species are genetically diverse (*e.g.* Dobzhansky, 1955) and the causes and maintenance of this diversity present problems of great importance in the study of evolution. This genetic diversity is, in some materials at least, so great that we can speak of genetic individuality. Its study is therefore also of general philosophic importance, especially as man himself is one of the species in which (apart from identical twins) each individual is genetically unique (Medawar, 1957).

Systems that promote outbreeding help to maintain such genetic diversity. Indeed we regard the maintenance of diversity as the main function of outbreeding systems and of all the relevant aspects of genetic systems that promote heterozygosity and segregation (Darlington, 1939). But outbreeding systems cannot of themselves maintain gene frequencies indefinitely, and it is necessary to postulate selective forces that will do so. The same selective forces may be supposed responsible for the maintenance of the genetic systems themselves.

Three such selective forces have been proposed. The first is long-term selection for adaptability or genetic flexibility (*e.g.* Darlington, 1939; Mather, 1943; Thoday, 1953). Organisms have evolved in a changing environment, and selection must have, in the long run, eliminated those forms which did not maintain sufficient genetic flexibility. At the same time, short-term selection promotes genetic stability, so that high mutation rates which would permit genetic flexibility only at the expense of stability are inadequate. Heterozygous systems permit both stability and flexibility, so that stable heterozygous systems and the genetic diversity they bring about would result. Such long-term selection seems sufficient explanation to some but others doubt whether the selective forces could be adequate to account for the prevalence of outbreeding systems or the degree of diversity in contemporary populations. This quantitative objection may be valid, though it is difficult to assess, for selection against genetic inflexibility must in the long run be absolute, and the capacity of pathogens for rapid evolution implies that the long run may be shorter than we think. Nevertheless it does seem probable that other factors must be involved.

The second selective force is selection for heterozygotes. Here it is supposed that heterozygosity *per se* has some intrinsic virtue, in providing a more complex and versatile physiology. This must derive from inter-allelic interactions, or otherwise duplication could permit both alleles to become homozygous. There is evidence suggesting that such heterozygous advantage may occur, though it is always difficult to be sure what " allele " means in this context. The most cogent evidence is that of Allison (1955) and Hunt and Ingram (1958) concerning sickle-cell anaemia and the chemical structure of the haemoglobins. Here, however, there seems no good reason for invoking inter-allelic interaction. The evidence rather indicates

independent action of the two alleles and there seems no reason why duplication should not ultimately occur and produce a homozygous individual capable of producing haemoglobin A and one or both the alternatives. On the whole there seems little reason for supposing that heterozygosity had any primitive advantage (Thoday, 1955). Though some results (e.g. Wallace and Vetukhiv, 1955) are difficult to explain, it seems unlikely that superior fitness of heterozygotes can be the prime cause of heterozygosity. The success of haploid and inbreeding species argues strongly against heterozygosity having any essential virtue other than as the prerequisite of segregation as Mather has made clear (*e.g.* Jinks and Mather, 1955).

The third type of selective force (Levene, 1953; Moree, 1953; da Cuhna and Dobzhansky, 1954; Mather, 1955*a*; Li, 1955; Robertson, 1956; Thoday, 1956) is one which may be supposed to operate in the short run to maintain the frequencies of heterozygotes. This is continued selection for actual phenotypic (especially physiological) diversity. Most populations occupy quite heterogeneous environments and are therefore exposed to disruptive selection. Their environments are also subject to cyclic changes so that the populations will also be exposed to cyclic selection which is likely to have similar effects. Robertson (1956) has shown theoretically that disruptive selection (D^- in type) would be expected to maintain gene frequencies and that stabilising selection would be expected to lead to fixation. The present experiments show that this result is borne out in practice. Further, the D^+ line, which represents a situation likely to occur in nature more often than disruptive selection with negative assortative mating, shows that disruptive selection can actually increase the effective variation within a population. The experiments therefore demonstrate that heterogeneity of the environment can in practice promote genetic diversity, as well as maintaining such diversity, and provide evidence favouring the view that disruptive selection is an important cause of the genetic diversity which we find in populations in nature.

9. SUMMARY

1. Three lines, each derived from the same base stock of wild *D. melanogaster*, have been maintained under different systems of selection for sternopleural chaeta-number. One (D^-) was maintained under disruptive selection with negative assortative mating, one (D^+) under disruptive selection with positive assortative mating, and the third (S) under stabilising selection.

2. D^- selection resulted in an increase of mean chaeta-number, some deterioration of developmental stability (homeostasis) as measured by sternopleural asymmetry, but little if any change of variance that could not be attributed to the correlation of variance and mean.

3. D^+ selection resulted in an increase of variance.

4. S selection resulted in a decrease of variance and a decline of vigour.

5. The D lines were more responsive to directional selection than the S line.

6. The D^+ line has become more responsive to directional selection than the base stock from which it was derived. The S line is less responsive than the base stock.

7. Estimates of realised heritability are D^- 0·29, D^+ 0·26, the base stock 0·15, and S 0·09. The D^+ estimate is minimal as two of the three tests on which it is based were carried out at unfavourable times in the history of the line.

8. It is concluded that disruptive selection can promote and stabilising selection can decrease genetic flexibility, and, therefore, that heterogeneity of habitat may be an important cause of genetic diversity in natural populations.

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EFFECTS OF DISRUPTIVE SELECTION

II. POLYMORPHISM AND DIVERGENCE WITHOUT ISOLATION

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1. INTRODUCTION

EVIDENCE that supports the view that disruptive selection can promote genetic diversity within a population has been presented in a previous paper (Thoday, 1959). Disruptive selection, resulting from heterogeneity of environmental conditions, can therefore be regarded as a significant cause contributing to the genetic diversity that is such a striking property of natural populations of outbreeding species.

Mather (1955) has argued that disruptive selection, when the different forms selected are interdependent, may be expected to produce polymorphic populations, and the evidence already put forward helps to support this argument in so far as it is legitimate to regard polymorphism as but a special extreme form of this general genetic diversity. Mather also pointed out that only experimental study of disruptive selection can inform us of the relationship between selection differential and gene-flow, which must in part determine the quantitative importance of isolation in evolution.

One of the lines described in the previous paper (D^+) provides relevant information. Some of this information has already been published in a preliminary report (Thoday, 1958).

2. MAINTENANCE OF THE LINE

Details concerning the maintenance of this line under disruptive selection with positive assortative mating were given in the previous paper. It is only necessary to reiterate here that each generation was represented by four single-pair cultures, two of which were selected for high and two for low sternopleural chaeta-number (selection of one fly in 20). Each culture was assayed by counting 20 flies of each sex. The mating system was described in the previous paper in terms of the four component female sub-lines. It is recast in terms of the four male sub-lines in table 1 of this paper as this renders it more readily intelligible from the present point of view. There are two high male-lines and two low male-lines. Each selected high male is mated in each generation to a female selected for high chaeta-number but taken from a culture of a low male-line. Likewise a low male is mated to a low female from a culture of a high male-line.

There are therefore in each generation two high and two low cultures, but crossmating ensures that they are all parts of the same

population, which is subjected in every generation to selection for both extreme chaeta-numbers.

The population can also be considered in terms of its component high and low sub-populations. These are subjected to divergent directional selection but are not isolated in any way. On the contrary there is between them " forced gene-flow," for all flies are progeny of crosses between the two sub-populations. One-half of the autosomal genes and, as migration from one sub-population to the other is through females, two-thirds of the X-linked genes are exchanged in each generation. Only the differential segments of the Y chromosomes are isolated. The mean chaeta-numbers of the two sub-populations may therefore be used to test the efficacy of this type of disruptive

TABLE 1

Mating and selection system

Parents of Generation	Culture: *i.e.* male sub-line			
	1(H)	2(H)	3(L)	4(L)
	♀ ♂	♀ ♂	♀ ♂	♀ ♂
n	$H_3 \times H_1$	$H_4 \times H_2$	$L_1 \times L_3$	$L_2 \times L_4$
n+1	$H_4 \times H_1$	$H_3 \times H_2$	$L_2 \times L_3$	$L_1 \times L_4$
n+2	$H_3 \times H_1$	$H_4 \times H_2$	$L_1 \times L_3$	$L_2 \times L_4$
n+3	$H_4 \times H_1$	$H_3 \times H_2$	$L_2 \times L_3$	$L_1 \times L_4$

The entries designate the flies used to produce the culture in the generation shown in the first column. H indicates the highest and L the lowest chaeta-number fly found in the appropriate culture. 1, 2, 3, 4 indicate the culture from which the fly was taken.

selection, and to test whether maximal gene-flow can prevent divergent selection pressures from causing the two sub-populations to diverge.

As reported earlier, an error of selection was made in generation 21 when the whole population was selected for low chaeta-number.

3. RESULTS

(1) Divergence of mean chaeta-numbers

The differences between the mean chaeta-number of the two high cultures and that of the two low cultures are illustrated in fig. 1. The first generation of selection produced a positive divergence of mean, but in this generation, of course, there was no gene-flow. This generation is not really relevant to the experiment but sets a standard showing the responsiveness of the line to one generation of divergent directional selection. In the following generation the gene-flow between the two sub-populations was introduced and the difference of mean was reduced as a consequence. Thereafter it fluctuated widely, sometimes having negative values, until generation 9, after which there was a steady fall until generation 13. In this generation (13), however, some

change clearly occurred which permitted the population to maintain a consistently positive difference between its high and low components. This difference, though it fluctuated, was of the order of that produced by directional selection in generation 1. The difference was maintained until generation 21 when the selection for low chaeta-number in the whole population reduced it to zero. Further disruptive selection, however, rapidly restored the difference (at the expense of within-sex-and-culture variance which fell from 2·5 to 1·5 : see Thoday,

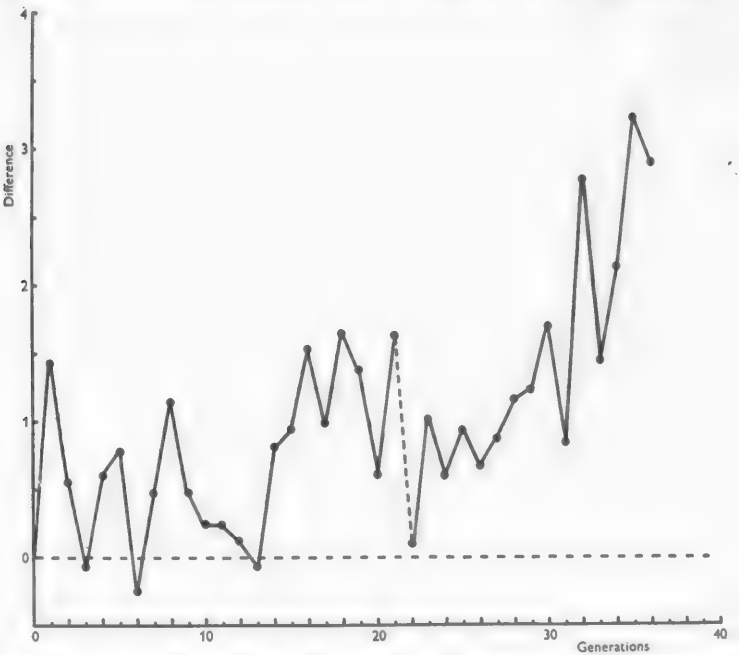


FIG. 1.—Differences, in chaetæ per fly, between the mean of the two high male-lines and the mean of the two low male-lines. The broken line indicates the error of selection at generation 21. (It should be noted that 9 must be added to the number of generations when this figure is compared with Figure 1 of Thoday, 1959).

1959). The difference has since increased (and the within-culture variance also : see Thoday, 1959), and is now at a level considerably higher than that attained in generation 1. The error in selection was unfortunate, but it at least serves to show that the population could recover from such an error.

(ii) The individual male-lines

Fig. 2 represents the deviations of each of the four male-lines from their joint mean and reveals information concerning the changes that have occurred.

It is clear from the figure that, until generation 14, there was no tendency for any one male-line to stand out consistently as High or Low. The highest male-line is more often H2 and the lowest male-line is more often L3, but no consistent behaviour of the different male-lines could be postulated. Since generation 13, however, L4 has been almost consistently the lowest of the four male-lines and H1 has been the highest except for a period, after the selection error in

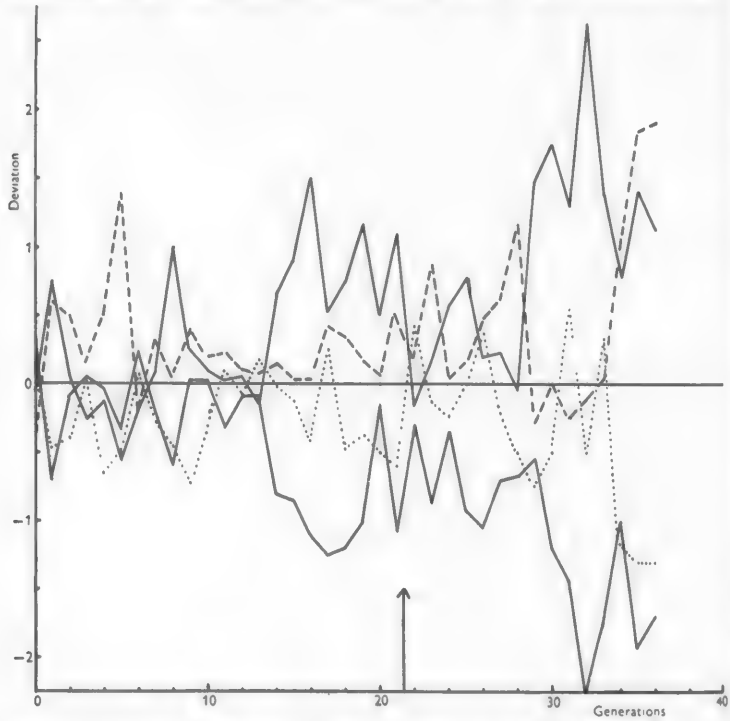


FIG. 2.—Deviations, in chaetæ per fly, of each male-line from their joint mean. Solid lines H1 and L4. Broken line H2. Dotted L3. The arrow indicates the selection error.

generation 21, during which H2 differentiated temporarily. H2 was consistently higher than L3 from generation 14 to 21 and from 23 to 28, but the order of difference between them was much less than that distinguishing H1 and L4, until generation 35 when H2 and L3 also differentiated strikingly.

The first significant event which led to the development of the consistent distinction of the high and low sub-populations therefore occurred at generation 13, in H1, or in L4, or in both. As it led to the immediate separation of both H1 and L4 from the other two male-lines, it clearly involved both the production of a high genetic factor in H1 and the production of a low genetic factor in L4.

The coincidental production of the two factors suggests that recombinations were involved, producing the two coupling products from a repulsion linkage of relevant genetic factors (*cf.* Mather, 1943). Thereafter (until the error was made in generation 21), these would be maintained as " effective factors " (Mather, 1949) in the two male-lines and could not break down in the absence of recombination in males. They could only with difficulty escape the male-line in which they originated, because selection of the migrating females would tend to eliminate them. In generation 21 the high factor would be lost from H1 when it was in error selected for low chaeta-number. Thereafter H1 did not really recover until in generation 28 a new event seems to have occurred, this time having effects of greater magnitude. After this second event H1 and L4 differed in mean chaeta-number considerably, but H2 and L3 had similar means. A third event seems to have occurred in generation 33 leading to the separation of H2 and L3. This third event will not be discussed here; it is too recent to be considered in the present analysis.

(iii) The differences distinguishing male-lines H1 and L4

General considerations would lead us to expect H1 and L4 to be distinguished by autosomal factors. It is conceivable that H1 and L4 might be distinguished solely by their Y chromosomes, but that this is not so is clear. Y chromosomes are usually confined to males and, if so, could only account for a distinction between the males of the subpopulations. But the observed differences that distinguish male-line H1 from male-line L4 occur in the females as well as the males. Females, of course, can carry Y chromosomes, but that the lines differ in supernumerary Y chromosomes seems unlikely. There has been no evidence, in crosses made with females from the lines, of sons with paternal X chromosomes such as occur when supernumerary chromosomes are present.

The cytoplasm is ruled out as a possible explanation by the design of the mating system and we must look to the other chromosomes for an explanation.

The X chromosomes can hardly be responsible. A brief consideration of the effect of the mating and selection system on a heterozygous X-linked locus shows that such heterozygosity could not maintain a consistent differential. Any X-linked allele or effective factor distinguishing a high (or low) male-line would be lost in one generation. X-linked effects would therefore have to be complex to explain the situation.

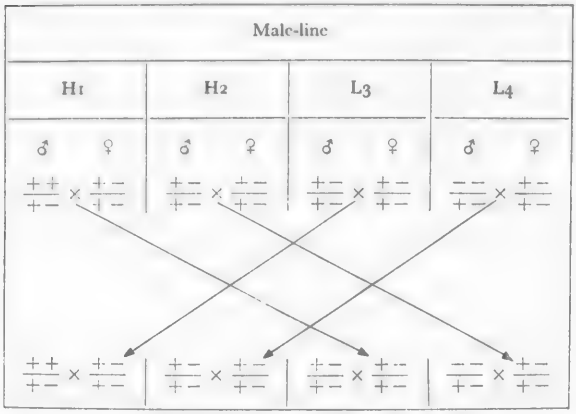
Autosomal factors could, on the other hand, readily provide an explanation. It has already been suggested that the high and low male-lines H1 and L4 were, until generation 21, distinguished by effective factors produced by recombinational events that converted repulsion complexes of genes into the corresponding coupling complexes. Such a system involving a pair of autosomes and assuming

only additive effects of the genes involved could well operate in the mating system by which the line is maintained as is indicated in table 2, which assumes homozygosity of the line except for the heterozygous " effective factors ".

This system could work even with the loosest possible linkage and with $-+$ as well as $+-$ intermediate chromosomes, for not only does lack of recombination in the male ensure its permanence, but also recombination, were it to occur, could not do more than reconstitute the existing chromosome types. It could therefore work

TABLE 2

Proposed simple model of the genetic system distinguishing male-lines H1 and L4. (*Compare mating system in table 1*)



equally well (and perhaps be set up more readily) in a population in which it was the males instead of the females who migrated from one side to the other. This system is formally equivalent to a three-allele polymorphism.

If such were the system operating in the population after generation 13, then of course the high " allele " will have been lost at generation 21. A new high allele seems to have been produced in male-line H1 in generation 28. If this were also an " effective factor ", produced by recombination, then it would not be likely to be " allelic " to its predecessor. If anything we might expect it to be in a different chromosome or chromosome arm.

The result would be a double test-cross system. H1 would be heterozygous for high and intermediate alleles at one " locus ", L4 would be heterozygous for low and intermediate alleles at another " locus ".

According to this view high chaeta-number flies taken from H1 should be heterozygous for a high factor and an intermediate factor, and low chaeta-number flies from L4 should be heterozygous for a

low factor and an intermediate factor, the high and low factors being non-allelic. Test crosses have been made to determine whether this is so.

(iv) Test-cross results

The test crosses were made in generation 33 using extreme high females from H1 and extreme low females from L4. These were mated to males, all with 18 chaetæ (9 on each side) selected from a slightly inbred stock homozygous for *y*, *bw* and *st*. Ten F1 males from the progeny of each of the four females were test-crossed to *y bw st* females, and the chaetæ were counted in 5 flies of each sex of each of the four classes of test-cross progeny, making 1600 flies in all.

The test crosses assess all three major chromosomes. Effects of II and III are tested by segregation of chaeta-number from the markers *bw* and *st*. Effects of the X (or supernumerary Y) chromosomes would be indicated by differences of sex difference, for the test-cross females contain an X from the tested male-line, whereas the test-cross males do not.

The test-cross data are summarised in fig. 3. Of the 20 L4 genomes tested, 11 were distinguished from the *y bw st* tester stock by a low chaeta-number effect attributable to chromosome II. Of the 20 H1 genomes tested, 4 were clearly distinguished from the *y bw st* stock by a high chaeta-number effect attributable to chromosome III. Two others, referred to as dubious in the figure, seemed as if they might belong to the same class, for they showed a smaller effect, also attributable to chromosome III. The remaining genomes are scattered round intermediate values for both chromosomes though, as must be expected, there are residual differences, attributable to both chromosomes, distinguishing the H1 and L4 genomes. No differences attributable to the X chromosomes emerged. A full analysis of variance of the *bw st* homozygotes obtained in the test cross gave no significant differences except the overall sex difference. Thus the differences illustrated in fig. 3 depend on the genomes extracted from H1 and L4 and not on variation of the autosomes from the *y bw st* standard against which the test was made. This analysis also shows that the sex chromosomes are not contributing to the difference between the H1 and L4 genomes tested.

The results of the test crosses show that half of the L4 genomes tested contained low chaeta-number second chromosomes. The L4 females tested were therefore heterozygous for a low and an intermediate chaeta-number factor on chromosome II.

The H1 females segregated high and intermediate third chromosomes, but there are fewer high segregants than the 10 expected, and it is not so clear that the H1 genomes fall into two distinct classes. Six of the derived chromosomes were therefore retested by test-crossing derived *y bw* +/*y bw st* males to *y bw st* standard females. Two of these derived chromosomes were of the high class, two were those of the dubious class, and two were of the intermediate class (see fig. 3).

Two cultures of each chromosome were obtained and 10 flies of each sex and genotype were counted in each culture (480 flies in all).

The results of this further test cross are summarised in table 3. The analysis of variance suggests that at least three classes of third chromosome were extracted from the H1 females, for each of the four degrees of freedom concerned with class means and with class × +/*st* interactions are associated with significant mean squares. Repre-

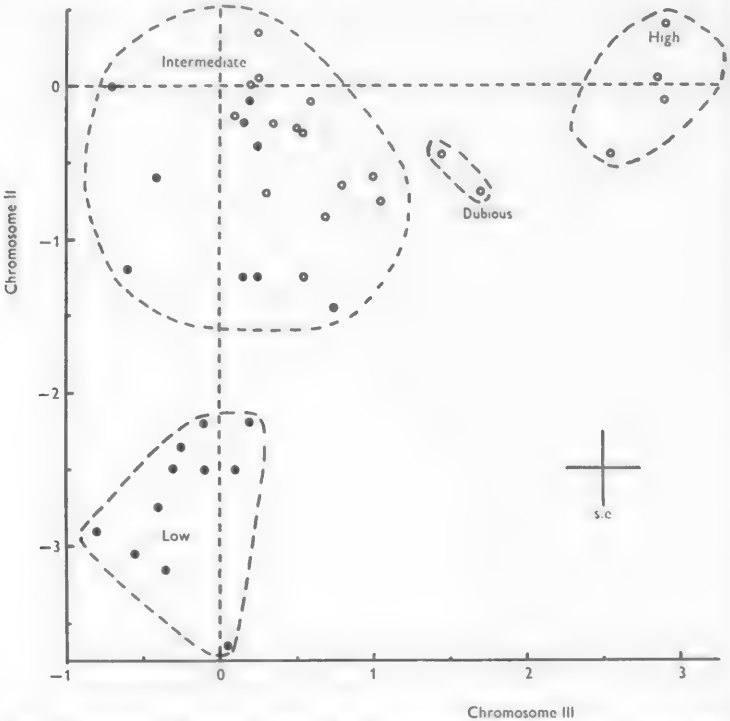


FIG. 3.—Deviations, in chaetæ per fly, from homozygous *y bw st* standards, produced by chromosomes extracted from extreme H1 and L4 females. Points L4 genomes. Circles H1 genomes. The mean standard error of a single entry is indicated.

sentatives of all three classes were obtained among the progeny of one of the two females originally extracted from H1. It thus seems that third chromosomes of the " dubious " class are recombination products in which the effective factor distinguishing H1 has partially broken down. This evidence supports the view that recombination rather than mutation is the key factor in the responses that occurred in the line, though it also indicates that the genetical model proposed on p. 210, is, as might be expected, too simple.

Despite the results for H1 being less clear-cut, the test crosses show unequivocally that L4 and H1 are heterozygous for genetic factors

giving the extreme chaeta-numbers, and allelic factors giving intermediate chaeta-numbers that can migrate from one part of the population to another without reducing the differences distinguishing the different male-lines. These factors, though they are probably linked

TABLE 3

Results of second test cross of H1 third chromosomes. (*Entries are the differences in mean chaeta-number between* +/st *and* st/st *flies. All flies homozygous* y *and* bw)

Chromosome tested	Class of Chromosome III		
	Intermediate (I)	Dubious (D)	High (H)
A	−0·1750	1·2250	2·4500
B	0·5250	1·1250	2·5250
Joint mean deviation	0·1750	1·1750	2·4875

Analysis of variance

Source	Mean Square	*n*	
H/(D+I)	22·8750	1	†
D/I	11·8125	1	*
+/*st*	196·3521	1	‡
Sex	46·2521	1	‡
Sex × +/*st*	3·5021	1	
HDI × sex	2·9646	2	
H/(D+I) × +/*st*	88·1667	1	‡
D/I × +/*st*	19·4374	1	†
HDI × +/*st* × sex	2·2145	1	
Cultures and chromosomes	1·0521	9	
Cultures and chromosomes × +/*st*	1·6354	9	
Other interactions	2·5632	18	
Individuals (error)	2·6891	432	

* $P<0·05$ † $P<0·01$ ‡ $P<0·001$

complexes, are behaving as genes. The population is therefore polymorphic.

The present indications are that further response of the population is likely to occur. This may involve the further development of the existing effective factors by further recombination, the development of additional effective factors, or the selection of a background that enhances the effects of the existing loci, perhaps by dominance modification. The line is being maintained and it should be possible to determine which of these means is used. At present there are no indications that selection of modifiers has been involved in the development of the polymorphism, for none of the relevant interactions in the test-cross data are clearly significant.

4. DISCUSSION

The results of this experiment are clear-cut. The two sub-populations of the line, though there is 50 per cent. gene-flow between them, have diverged significantly as a result of the divergent selection pressures imposed upon them. At first they were unable to diverge consistently, but after a time they developed a genetic constitution permitting them to do so. Continued selection has increased the difference distinguishing them.

This divergence has been made possible by the development of a low chaeta-number second chromosome factor in the low sub-population with an allele of intermediate effect, and of a high chaeta- number third chromosome factor in the high sub-population also with an allele of intermediate effect. The two sub-populations are able to maintain

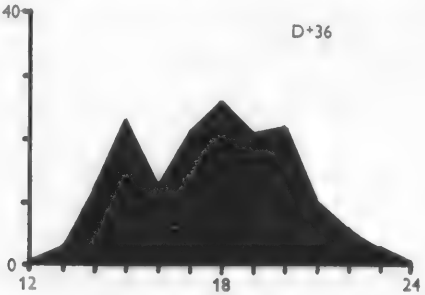


FIG. 4.—Distribution curve for the 160 flies assayed in generation 36.

a difference because the intermediate factors can migrate from one to the other without greatly affecting the difference of chaeta-number.

Whether the factors are genes in the more limited sense, or are relatively large chromosome regions (the evidence suggesting one of them can break down by recombination indicates the latter), they behave as genes in the system and are maintained at high frequencies by the selective pressures acting on the system. The population is therefore formally polymorphic even though, because these genes are influencing a continuously varying character, the population does not, as yet, show phenotypic discontinuity. Genetically the population is polymorphic. Even if it is not polymorphic by the strictest definition of Ford (1953), it is at least cryptomorphic in the sense of Huxley (1955). In fact the latest generation gives a bimodal distribution (fig. 4) which is significantly platykurtic, so that there is evidence suggesting phenotypic discontinuity.

It follows, therefore, that Mather (1955) was fully justified in his arguments that disruptive selection could in appropriate circumstances give rise to polymorphism. All that is required for the population to become strictly polymorphic is the selection of a background enhancing the effects of the " switch " genes.

It also follows that two component parts of a population can diverge despite lack of isolation barriers limiting gene exchange between them. The two pairs of cultures in each generation of this experimental line are, in a formal sense, in the same relative situations as would be the two parts of a population in a mosaic environment consisting of two distinct habitats arranged, for example, as a chequerboard. In such a situation a population could in principle develop two different forms, one adapted to each of the component environments even if there were forced (50 per cent.) gene-flow between the two forms (ecotypes). It is to be presumed that they could diverge more readily under random mating (25 per cent. gene-flow). They could do this by becoming polymorphic as the experimental population has done. It must be pointed out, however, that even if the individuals were mobile and therefore could choose to live in the environment to which they were adapted, the population would adapt to both environments at a cost. In the extreme situation envisaged a considerable proportion of the offspring would be intermediate and ill-adapted to both environments. Sessile forms would adapt in this way at greater cost as half the offspring would fall in the environment to which they were not adapted. However, the reproductive potentials of many species may leave a margin that would meet this cost, and habitats will often intergrade.

Biological "races" (*e.g.* Thorpe, 1956) are at the outset in situations quite similar to that under which the present population has diverged. The females remain and lay their eggs in the environment in which they were reared, having a pronounced preference for the species of host plant on which they have fed. By analogy with the present experimental population we must presume that, in these circumstances, two female lines on two host plants could diverge genetically in adapting to those host plants even if random mating occurred.

It is not suggested that this *is* the way in which genetic differences between biological races arise. It seems more likely that some measure of isolation would be involved, especially as there is a possibility that differing foods may of themselves cause isolation. Riley (1950) reared *Drosophila* on normal food and on food containing peppermint, and obtained some evidence that females not only laid eggs preferentially on the food on which they had been raised but also mated preferentially with males reared on the same food. If substantiated, this would involve an acquired isolation mechanism which could aid divergence of biological races.)

Nevertheless it *is* suggested that there is no *theoretical need* that there should be an isolation barrier before two such races may diverge genetically. Mayr (1942) clearly thinks there is such a theoretical need for he states that "it seems as if nothing would prevent the random mating by males, in other words, swamping. . . ."

The concept that, in the absence of isolating factors separating two components of a population, genetic differences between the two

components must be "swamped", derives from the concept of panmictic populations and from the mathematical consequences of panmixis. But that "interbreeding forms that are interfertile cannot diverge if they are able to breed together freely" (Carter, 1951), though it has become almost a text-book dogma, does not necessarily follow from the mathematics of panmixia, for we may have random mating of two kinds. Mating may be random with respect to the genotypes of zygotes, or it may merely be random with respect to the genotypes of adults. The first is panmixia but probably never occurs as natural selection will always ensure that the adults which mate are a non-random sample of zygotes. The second is not panmixia unless the adults represent a random sample of zygotes.

The statement that "interbreeding forms that are interfertile cannot diverge if they are able to breed together freely" is therefore not necessarily true, if, as it seems to do, it refers to adults. Neither would random mating by males necessarily lead to "swamping" as Mayr suggests. Selection operating between zygote and adult could ensure that random mating by males would not lead to swamping.

It is therefore in principle possible for biological "races" and ecological "races" to originate in the absence of isolating factors. If, thereafter, changed circumstances set a premium on further divergence of the forms or on more precise adaptation to the local environments (*cf.* Mather, 1943), selection for isolation could occur essentially as Dobzhansky (1941) has argued it will when two geographically isolated forms meet. This process would be sympatric speciation.

The same arguments raise the question whether Mayr (1954) is necessarily right in suggesting that the failure of peripheral populations of a species to adapt successfully to the local environment is explained "if we assume that this process of adaptation by selection is annually disrupted by the infiltration of alien genes and gene-combinations from the interior of the species range which prevents the selection of a stabilized gene-complex adapted to the conditions of the border region". There seems no reason in principle why a locally adapted population should not be formed even if one-way gene-flow into the local area were so great that all progeny were hybrids. The local individuals would need only to produce a dominant effective-factor conferring adaptation. A perpetual test-cross system would result and half their progeny by crossing with the migratory inflow would be adapted to the local environment. That it would be only half the progeny would set a premium on the subsequent development of isolation. Again the result could be speciation by a process in which divergence could precede isolation.

It may be objected that these arguments are not fully supported by the evidence put forward here, as, in the present population, the migrators are selected for characters appropriate to the sub-population *to* which they migrate. Though this may sometimes happen in nature, the reverse might happen more frequently. However, in nature the

maximum gene-flow will be that involved in random mating. Experiments now in hand indicate that two sub-populations under divergent selection pressures, with random mating permitted by migrators selected for characters appropriate to the sub-population *from* which they migrate, can also maintain genetic differences.

5. SUMMARY

1. Further results obtained with the population exposed to disruptive selection with positive assortative mating are described.

2. The population consisted of four male-lines, two selected for high sternopleural chaeta-number and two for low. Males of a high male-line were always mated to females from a low male-line selected for high chaeta-number. Males of a low male-line were mated to females from a high male-line selected for low chaeta-number. The population thus had a high and a low component, but the two were always crossed so that the gene-flow between them was maximal.

3. This gene-flow at first prevented the divergence of the two sub-populations, but after generation 13 they developed consistently and increasingly different chaeta-numbers.

4. Analysis of the data showed that for a long period only one high and one low male-line had diverged. The high male-line is distinguished by a factor in chromosome III and the low by a factor in chromosome II. The population is polymorphic. The factor in chromosome III can break down by recombination.

5. The line therefore demonstrates:

(i) that disruptive selection can produce polymorphism;

(ii) that two components of a population can diverge without any isolation limiting gene-flow between them.

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THE EYE-PIGMENTARY SYSTEM OF *DROSOPHILA*

VII. THE WHITE LOCUS

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1. INTRODUCTION

THE white locus of *D. melanogaster* (1·5 in the X-chromosome) is of great importance for the study of allelism since it has mutated to a large series of multiple alleles, although these are also thought to consist of at least two series of pseudoalleles. The function of this locus has been discussed (Beadle and Tatum, 1941; Nolte, 1952) and it is thought that the probable mode of action of the wild-type allele is at the level where a common substrate is differentiated for the formation of two specific substrates that combine with the red and brown chromogens respectively, to form the red and brown chromophore groups. In this investigation it was hoped to gain further information on the relations between mutation and the functions of a gene. Two types of genetic differences were studied. First, fourteen mutant genes in the multiple series, *i.e.*, satsuma (w^{sat}), coral (w^{co}), wine (w^{w}), coloured (w^{col}), blood (w^{bl}), cherry (w^{ch}), eosin (w^{e}), eosin^2 (w^{e2}), eosin^3 (w^{e3}), apricot (w^{a}), apricot^3 (w^{a3}), apricot^4 (w^{a4}), tinged (w^{t}) and white (w), and second, two position effects of the mottled type, *i.e.* white-mottled-4 (w^{m4}) and white-mottled-5 (w^{m5}) The methods applied in this study have been fully described (Nolte, 1952, 1954*a*).

2. OBSERVATIONS

(*i*) *Eye-colour phenotype*

These mutants are described by Bridges and Brehme (1944) and some have been described in a previous study (Nolte, 1950). The allele w^{e3} was found to be a new allele after photometric analysis, although it had originally been extracted from a white-eyed strain and had been accepted as the usual eosin allele (Nolte, 1944). The female has a slightly darker eye-colour than the w^{e} female and the male is slightly pinker than the w^{e} male. The two alleles w^{co} and w^{w} are apparently indistinguishable phenotypically (Bridges, 1938) but have been found to differ in pigment content. The position-effect w^{m5} is more variable in eye-colour than w^{m4} (Nolte, 1950), generally showing mottling of garnet with colourless patches, and at reduced temperatures the eye is highly speckled.

(ii) Histology

Microscopically the white series of alleles seems to belong to the brown type of eye-colour mutants (Nolte, 1950). The pigment cell regions (primary, secondary, basal and post-retinal) in the eyes of females appear as follows. The mutant w^{ea1} resembles the mutant brown (*bw*), the primary cells have a yellowish tone due to brown granules, while the other three regions have a purplish-brown colour in aggregate caused by brown granules; the granules appear to be small in size as in the mutant *bw*, the basal cells are smaller than normal, and the post-retinal region appears to be normal in extent. The pigment regions of the allele w^{col} have in aggregate a bright brown colour caused by medium brown granules throughout all four regions, these granules being indistinct in outline or smaller than normal; the basal pigment cells are small and about as compact as in w^{co}, while the post-retinal region is narrower than normal. The two alleles w^{co} and w^{w} resemble each other in histological eye-colour but the former is somewhat darker; they show less colour than the allele w^{col} but with a darker tone of brown, with the exception of the post-retinal region which is more purplish-brown. The granules, excepting those in the primary cells, are indistinct in outline and the basal cells are small rounded masses. The allele w^{bl} at 20° C. has primary cells with brown granules, the other pigment regions being a reddish-brown as in the mutant carmine (*cm*) (Nolte, 1950); all granules are more distinct in outline than in the other alleles, and the basal cells are more nearly normal in size. At 25° C. the colour is paler and more brownish, the granules being indistinct in outline excepting in the primary cells and the basal cells are very small rounded masses. The allele w^{e2} has a yellowish tinge to the brown of the primary cells and a more reddish- or purplish-brown in the other three regions; the granules are less indistinct than in w^{co}, and the basal cells are small. In w^{a3} the aggregate colour is a medium brown, and the granules in all regions vary in size, many being larger than normal; the basal cells do not show such compact masses of granules as in the other alleles. The allele w^{e} resembles w^{co} in all respects with the exception that it is much lighter in colour; and the primary cell granules are very distinct; the allele w^{e3} is very similar to w^{e}. In w^{ch} there is a similar picture, but the primary cell granules are not quite as distinct. The alleles w^{a} and w^{a4} are very light in eye-colour, and in w^{t} and w no colour can be detected histologically.

Like w^{m4} (Nolte, 1950) the position-effect w^{m5} also shows a very variable histological picture, the colour in aggregate being brown with varying numbers of ommatidia or parts of ommatidia unpigmented, but with a lesser number of the elements of an ommatidium pigmented than in w^{m4}, though the pigmented sector runs throughout all four pigment regions. The basal cells seem smaller or more compact than normal, while the post-retinal region has a reduced number of pigment cells visible.

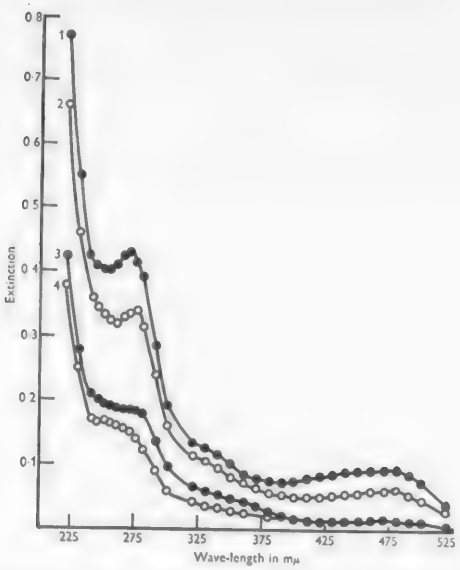

FIG. 1.—Light-absorption curves of the red pigment extracted in AEA, for 10 heads per 1 c.c., in the whole range. 1, blood (w^{bl}) at 20° C.; 2, eosin[2] (w^{e2}) at 25° C.: 3, blood at 25° C.; 4, apricot (w^{a}) at 25° C.

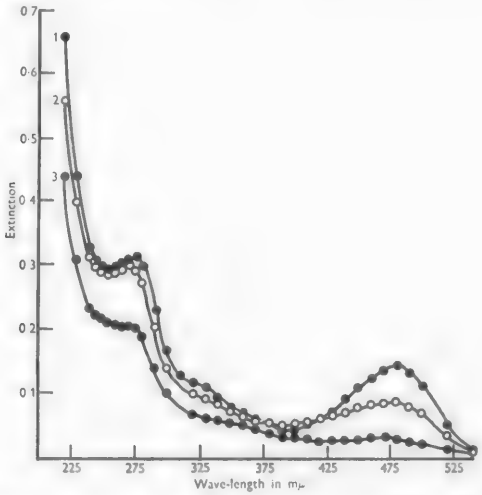

FIG. 2.—Light-absorption curves of the red pigment extracted in AEA, for 10 heads per 1 c.c. 1, coloured (w^{col}); 2, the heterozygote coloured-wine (w^{col}/w^{w}); 3, wine (w^{w}).

(iii) Photometric analysis

All the strains were examined for types of pigment, and so also were certain compounds with other mutant genes; some of the light-

TABLE 1

Relative amounts of red and brown pigments in the eyes of the multiple alleles at the white locus

Allele	Red pigment	Brown pigment
w^+	0·8993±0·0052	0·0866±0·0007
w^{ce1}	0·1454±0·00220	0·0182±0·00043
w^{ce2}	0·0616±0·00110	0·0506±0·00051
w^{a3}	0·0542±0·00084	0·0091±0·00025
w^{co}	0·0496±0·00042	0·0302±0·00027
w^{sat}	0·0321±0·00047	0·1083±0·00140
w^{w}	0·0308±0·00054	0·0342±0·00044
w^{a3}	0·0240±0·00038	0·0290±0·00078
w^{bl}	0·0188±0·00060	0·0122±0·00020
w^{e}	0·0142±0·00020	0·0182±0·00034
w^{ch}	0·0125±0·00020	0·0285±0·00046
w^{a}	0·0108±0·00018	0·0089±0·00020
w^{a4}	0·0100±0·00027	0·0085±0·00016
w^{t}	0·0034	0·0028
w	0·0024	0·0020

absorption curves of pigment extracts are given in figs. 1-4. The amounts of the red and brown eye pigments were determined for 10-12 cultures of each strain, the red pigment being absorbed in acidified

TABLE 2

Relative amounts of red and brown pigments in the eyes of w^{bl}, w^{m4} *and* w^{m5} *at different temperatures*

Strain	Temperature	Red pigment	Brown pigment
w^{bl}	18° C.	0·2012±0·0056	0·0553±0·0016
	20° C.	0·0950±0·0038	0·0435±0·0008
	21° C.	0·0705±0·0026	0·0372±0·0018
	24° C.	0·0255±0·0008	0·0183±0·0005
	25° C.	0·0188±0·0006	0·0122±0·0002
w^{m4}	20° C.	0·5516±0·0226	0·0691±0·0021
	22½° C.	0·7039±0·0039	0·0757±0·0001
	25° C.	0·8310±0·0212	0·0845±0·0001
w^{m5}	19° C.	0·0662±0·0067	0·0209±0·0011
	22½° C.	0·1593±0·0243	0·0299±0·0010
	25° C.	0·2248±0·0202	0·0347±0·0018

30 per cent. ethyl alcohol (AEA) and the brown pigment subsequently in acidified methyl alcohol (AMA). The amounts of pigment in extracts of the various strains are given in tables 1-3, the data being given as E at 480 mμ for the AEA extract and at 444 mμ for the AMA extract, for concentrations of 10 heads per 1 c.c. solvent.

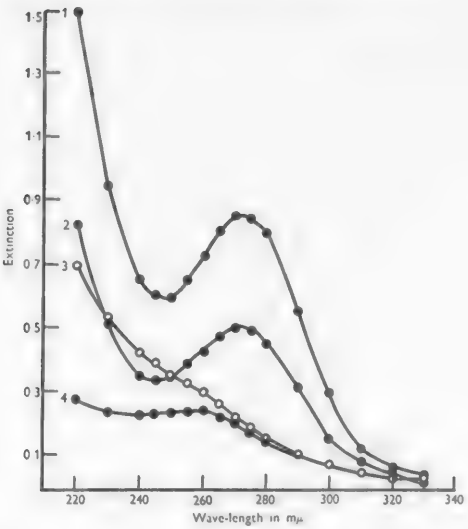

FIG. 3.—Light-absorption curves of the brown pigment extracted in AMA, for 10 heads per 1 c.c. 1, wine (w^w), reduced extract; 2, apricot (w^a), reduced extract; 3, wine, oxidized extract; 4, wine, untreated extract.

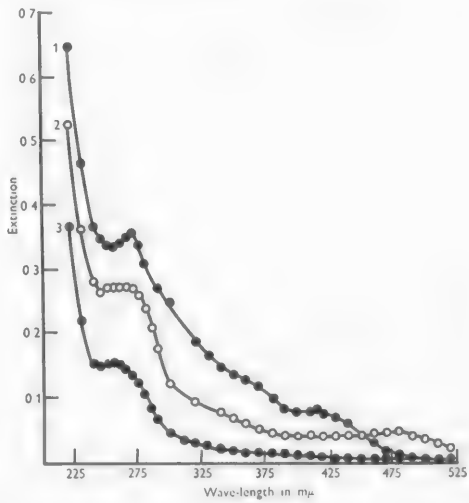

FIG. 4.—Light-absorption curves of the red pigment extracted in AEA, for 10 heads in 1 c.c. 1, the combination eosin-sepia (w^a; *se*); 2, the combination wine-cardinal (w^w; *cd*); 3, the combination eosin-carnation (w^a; *car*).

3. DISCUSSION

The findings on the mutant changes at the white locus have yielded facts which are pertinent to the study of the nature of mutation as well as to that of the action of genes in general.

(1) In general mutants with mainly the brown pigment present in the eye appear to have small granules (Nolte, 1950, 1952), and such is the case with the alleles w^{sat}, w^{w}, w^{e} and w^{ch} in the present study. The brown pigment being deposited first and the amount being relatively small in comparison with the amount of red pigment in the wild-type, it would appear that the main increase in size of the granule is by an accretion due to deposition of red pigment. This seems

TABLE 3

Relative amounts of red and brown pigments in the eyes of certain mutants and their combinations with alleles of white

Strain	Red pigment		Brown pigment
	415 mμ	480 mμ	
w^{w}/w^{col}		0·0872	0·0242
w^{a} *car*		0·0045	0·0035
car		0·2829	0·0380
w^{e}	0·0189	0·0142	0·0182
w^{e} *se*	0·0810	0·0120	0·0390
se	0·3708	0·0556	0·1233
w^{w}		0·0308	0·0342
w^{w} *cd*		0·0457	0·0033
cd		0·9672	0·0131
w^{+}		0·8993	0·0866

proved by the presence of more distinct (or larger) granules in w^{bl} at 20° C., also in w^{e2} and w^{a3} which all have a higher red pigment content than the allele w^{co}, which has smaller granules. An exception is the granules in the primary cells which, in all the alleles, are more distinct and larger than the granules in the other pigment regions, and in all the alleles these granules appear to contain brown pigment only as in the mutant ruby (*rb*) (Nolte, 1950). In the allele w^{a3} the granules vary in size, with some larger than normal and with perhaps a reduction in number. It further appears that the size of the basal pigment cells, as judged by the masses of pigmented granules, may be based on the size of these granules; in w^{bl} at 20° C. these cells or their pigmented contents seem to be nearest to normal in size, while in the same allele at 25° C., with much smaller amounts of both pigments, these pigmented masses appear to be very small, as also occurs in other alleles with small amounts of pigments. However, there appear to be exceptions in w^{co} and w^{e2}, which possess comparatively large amounts of both pigments and yet in which the basal cells appear as small compact masses, though slightly larger in w^{e2} which has more pigment.

A consideration of all these facts leads to the conclusion that the alleles of white do not have as primary function the building up of the pigment granule, but that they rather affect its size secondarily through the amounts of pigment produced.

(2) The red pigment extracted by AEA in the wild-type and most mutants has been shown to have a light-absorption valley at 260 mμ and a peak at 275 mμ in the ultra-violet, as also a peak at 480 mμ in the visible range (Nolte, 1954*a*). A modified form of this pigment has been described for the mutants sepia (*se*) and clot (*cl*), *i.e.* a yellow pigment with the three points of absorption at 255, 270 and 415 mμ respectively (Nolte, 1954*b*). The photometric curves of the AEA extract of some of the alleles of white in figs. 1-2 appear to indicate the presence of a second modified form of the red pigment. The colour of these extracts varies from a very light to a darker yellowish tone and this is also reversibly reduced to a colourless state by sodium hydrosulphite, indicating that some of the properties of the pigment have not been altered. A comparison of the spectrophotometric curves of AEA extracts brings to light an interesting point, viz., that for the alleles with the greatest amount of this pigment these curves more nearly coincide with the curve of the normal red pigment (Nolte, 1954*a*); for example, for the alleles w^{e2}, w^{a3}, w^{c0} and w^{col} the three distinctive points of absorption are at 260, 275 and 480 mμ respectively. In fig. 2 it will be seen that the curve for the heterozygote w^{col}/w^{w} shows values between those of the two homozygotes. In general the AEA curves of alleles with low amounts of red pigment show that reduction in amount is correlated with a levelling-off of the ultra-violet peak of absorption and also of the peak at 480 mμ, though in several alleles the latter peak seems to be shifted back to 460-470 mμ.

AEA extracts of body fluids were made in order to test the presence of other substances dissolved from the head, and for this purpose the thorax was taken; for volumes more or less equivalent to those of the heads used for eye extracts an average E was calculated. For these fluids a valley appears in absorption at 237 mμ and a peak at 255 mμ, indicating the presence of a dissolved substance chemically different to the eye pigment. If the E values for this substance, presumably present in the body fluids, are subtracted from the data for the eyes it appears that for most of the alleles the three distinctive points of absorption are similar to that of the normal pigment. The data for an extract of the heads of a compound mutant with a low red pigment content, i.e. *rb car*, show light absorption similar to that obtained for some of the lower alleles of white. It thus appears probable that in the lower members of this series of alleles the absorption curves are modified by the relatively high fraction of other materials extracted from the head fluid by AEA, so that actually no modification of the red pigment has occurred. This conclusion invalidates that formed in an earlier investigation (Nolte, 1952) and also the conclusion of Ephrussi (1945) on the white alleles, *i.e.* that in the various alleles

various degrees of condensation of identical pigment molecules occur; it must, however, be noted that Ephrussi worked with the alleles made homozygous for scarlet (*st*), that he found a flattening of the steeply sloping curve for w^{co} in the visible region with increase in pigment deposition, and that he noted that the absorption curves have a distinct change of slope at 480 mμ. Maas (1948) working with white alleles homozygous for cinnabar (*cn*) did not reach the same conclusions as Ephrussi.

During the course of the present study it was discovered that the method of oxidizing or reducing the AMA extract of brown pigment affects the shape of the absorption curve in the ultra-violet, due to specific absorption by an excess of hydrogen peroxide and by some secondary product formed by the sodium hydrosulphite. This fact necessitates a modification of some conclusions which have been reached on the relationship between the red and brown pigments (Nolte, 1954*a*). Fig. 3 shows some of the effects of the oxidizing and reducing agents even in pure AMA, while the untreated AMA extract shows the type of absorption for the brown pigment. Though untreated extracts of brown pigment from the eyes of different alleles of white do not show absorption curves with such a clear-cut valley at 230 mμ and peak at 260 mμ as was found in the case of pupal eyes (Nolte, 1954*a*), it is possible that in adults these points are to some extent masked by the simultaneous extraction of increased amounts of impurities. In a previous study (Nolte, 1952) it was postulated that, because of a similarity in their peaks of absorption in the ultra-violet, the red and brown pigments had a part of their chromophore groups in common, and it was suggested that this part was produced by the action of the white locus. The findings of the present study do not substantiate this view, though such a part in common may still exist because the apparently partially oxidized state of the extracted brown pigment may have caused obliteration of double-bond structure and therefore loss of peak in ultra-violet absorption. A chromophore system may be very complex and absorption peaks produced in the ultra-violet would be summations of the effects of the partial chromophores. The conclusions arrived at, therefore, cannot be more explicit than that the white locus, in differentiating a common substrate, may cause the production of two constituents which may differ rather widely in regard to their spectrophotometric properties.

(3) An evaluation of the quantitative relationships between the multiple alleles and position effects may be arrived at by inspection of the data of tables 1-2; it should be noted that in a previous study (Nolte, 1952) the allele w^{co} was taken as identical with w^{w}. As point of reference is taken the amount of pigment occurring in the allele w^{bl} in which the content of both pigments is inversely correlated with rise in temperature. For the red pigment it was found that the increase in content is geometric, amounting to a factor of about 1·4 for each degree drop in temperature, while for the brown pigment the increase

is arithmetic, rising by an amount of about one-half of the amount present at 25° C. for each degree drop in temperature. Theoretically, if the curves of these increases are prolonged to 13° C., the amount of red pigment should be about 0·91 and of brown pigment about 0·086, *i.e.* approximately the amounts present in the wild type. Tests to confirm this showed that a critical temperature exists probably at about 15-18° C., at which the increase in pigment content with drop in temperature ceases due to metabolic upsets at the low temperature.

In comparing the quantitative relations between the various alleles of white two facts should be noted: first, the various strains are not isogenic for any series of genes that might affect the total amounts of the two pigments; secondly, the decrease in red pigment content is not correlated with decrease in brown pigment content for any specific allele. Comparing the red pigment content with that for w^{bl} at 25° C., the increase in the series is by a factor of approximately 1·2, *i.e.* the members of the series increase in content by a ratio approximately equal to that for w^{bl} for a drop of half a degree in temperature. For the brown pigment the increase is in an arithmetic series, rising by an amount approximately equal to that for w^{bl} for a drop of one degreee in temperature. Some remarks must be made in regard to these general rules. First, some alleles are grouped together for pigment content, e.g., w^{co}, w^{e3} and w^{ch} as a group with an equal content of brown pigment. Secondly, some gaps appear such as between w^{col} and w^{e2} for the red pigment, and between w^{sat} and w^{e2} and between w^{e2} and w^{w} for the brown pigment. Thirdly, the lower alleles do not seem to conform to these rules. For the higher alleles, however, the gaps could be accounted for if it could be predicated that these could be filled by alleles not easily distinguishable from those already known, in the way that coral and wine are not distinguishable in gross eye-colour.

Some kind of quantitative relation is therefore indicated as resulting from mutations at the white locus, and this relationship could support either an hypothesis for two differential functions of one locus or for the separate functions of two loci. Some evidence has been advanced for the hypothesis that the locus can actually be split into two, perhaps coinciding with the double band 3C2·3 in the cytogenetic map. In one investigation on crossing-over MacKendrick and Pontecorvo (1952) give evidence for crossing-over having taken place between w and w^{bl}, and between w and w^{co}. In the second instance Lewis (1952) has evidence of crossing-over between w and w^{a}, though implicit in this investigation is the hypothesis that if the two mutant genes are at different loci, a mutation at one locus blocks or impairs the functioning of the normal allele at the other locus when these two are in the same chromosome, i.e. $a+/+w$ has the mutant phenotype, a case of position pseudoallelism; these pseudoalleles may represent an established duplication of a single ancestral gene. Lewis assumes a simple model of one gene controlling the reaction A⟶B and the

other B⟶C, so that the position-effect can be assumed to result from the failure of substance B to diffuse readily from one chromosome to the other. If the white locus could be subdivided into two loci, a mechanism more acceptable of one gene having one product could be visualized for the differentiation of a common substance into two specific substrates; the two loci could respectively condition the reactions for the red and brown substrates. Thus for the genotype $a+/a+$ (apricot female) the mutant *a* would lower the production of one substrate and impair the function of the normal allele of the other locus; for the genotype $+e/+e$ (cosin female) the converse effect should then appear since in both mutants there is a reduction in both pigments. In such an event, however, the interaction would not be a simple one-way type as postulated by Lewis but rather a competitive relation (*cf.* the excess of brown pigment in $w^{ea\,t}$). Some indications of possible evidence for the postulate of Lewis do exist. First, such a scheme may account for the fact that the alleles arranged in two series for descending order of red and brown pigment content do not coincide. Secondly, Lewis advances the hypothesis that the genes eosin and white belong to one locus since they are lightened by the modifier Pale, whereas apricot is darkened by the latter. Thirdly, if the alleles are divided into two groups according to which sex has the darker eyes, we find those with the male darker are: coral, wine, coloured, blood and apricot, while those with the female darker are eosin, cherry and white. In connection with a system of grouping Mainx (1938) noted that compounds such as coral/wine and blood/wine are intermediate in phenotype while those such as eosin/blood and apricot/white more nearly resemble the darker homozygote. It is also noteworthy that the first group, with the exception of wine, have relatively more red than brown pigment, while the second group, with the exception of eosin, have relatively more brown than red pigment.

In the two mottled position-effects the amounts of both pigments increase with a rise in temperature, that for red being more rapid than for brown. For both pigments the rate of increase is arithmetic so that it must be assumed that the action of the position-effect is different to that of a mutation at the locus.

(4) With the effect of temperature on the pigment content of the allele w^{bl} as point of reference, some conclusions may be drawn on the effect of mutation on the function of this locus. For this allele Ephrussi and Herold (1945) determined the critical period for qualitative variation at 40-48 hour after puparium formation, that is, before pigment is visible in the eye. Presumably the actual critical period is very short and during it the differentiation of the assumed substrates is initiated and reaches a maximum. Decrease in temperature increases the duration of this period and consequently the enzyme activity of the gene w^{bl} is allowed additional time in which to continue its work. Basically, therefore, the mutation to this allele reduces the activity of

the locus, perhaps by reducing the amount of enzyme involved; in a similar way mutation to other alleles results in a reduction of activity —the production of hypomorphs. A correspondence exists between the rate of increase in pigment content in w^{bl} with decrease in temperature, and the rate of increase in the multiple alleles arranged in series; the members of the series do not, however, retain the same order for the red pigment as for the brown. The white locus does not, therefore, appear to act through a simple splitting of a substance into two since then these hypomorphic mutants should show an equivalent decrease in both pigments. An alternative hypothesis is that of Beadle and Tatum (1941), *i.e.* the locus may control an enzyme catalysing basically similar reactions in two parallel chains. On the other hand, the action of the white locus may indeed be through the differentiation of two enzymes from one precursor if it is accepted that other genes may take part in the production chains of the substrates differentiated for the two pigments, *e.g.* the loci of karmoisin (*kar*) and cardinal (*cd*) in the brown chain and the locus of purple (*pr*) in the red chain (Nolte, 1954*a*, 1955). The excess of red pigment in the mutant *cd* and of brown pigment in the mutant *pr* may be due to the diverting of part of the basic substance to the alternative chain. The quantitative data show that the red pigment increases more rapidly than the brown, although the brown is fully deposited before the red; thus if mutations at the white locus result in changes in the velocity of reactions controlled by this locus the balance of reactions in the two chains could be disturbed, resulting in differences in the relative amounts of the two final products.

The position of various genes in the scheme of eye-pigmentary reactions as proposed in previous studies of this series may be tested by reviewing some data on their combinations with alleles of white (table 3; fig. 4). The combination of w^e with carnation (*car*) shows large sub-additive effects for both pigment quantities; the mutant *car* delivers some substance in reduced quantities for further elaboration, and by the action of the hypomorphic mutant at the white locus even lesser quantities of the final products result. The combination of w^w with *cd* shows a large sub-additive effect on the brown pigment but an additive effect of the red pigment—it has been postulated that *cd* diverts some of the substrate from the brown to the red pigment pathway. The combination of w^e with *se* shows the epistatic effect of the latter in producing a modified red pigment and in having an additive effect on the brown pigment; the normal allele of *se* has been placed in the production chain of the common precursor required for the action of the white locus (Nolte, 1954*b*).

4. SUMMARY

The sixth group of eye-colour mutants of *Drosophila melanogaster* to be studied is the series of multiple alleles at the white locus, these

being w^{sat}, w^{co}, w^{w}, w^{col}, w^{bl}, w^{ch}, w^{e}, w^{e2}, w^{c3}, w^{a}, w^{a3}, w^{a4}, w^{t}, w and also the position-effects w^{m4} and w^{m5}.

1. The eye-colour phenotypes vary from white to very dark ruby. Histologically the colour results from granules which generally are brown, with little red; the primary pigment cells contain brown pigment only. The pigment granules are generally very indistinct excepting in the primary cells; in the higher members, however, the granules are more distinct or larger, and in w^{a3} a scattering of granules larger than normal occurs. The basal pigment cells are generally small compact masses. All these are effects of the slowing down of pigment production.

2. The red pigment of the higher members of the series yield spectrophotometric curves identical with that of the normal red pigment; for those with low red pigment content the curves are modified due to the relatively high admixture of impurities in the extracts. The brown pigment is also identical with the normal. For most alleles the untreated brown pigment extract gives an ultra-violet peak at 260 mμ (as for all pupal eyes) or at least a more rapid drop at this point.

3. The red pigment content of the allele w^{bl} increases geometrically with a drop in temperature by a factor of about 1·4 per 1° C.; the brown pigment content increases arithmetically with a drop in temperature by about half of that present at 25° C. per 1° C. If the alleles are arranged in separate series for increase in amount of the two pigments, these two series do not coincide. In the red series the increase is by a ratio approximately equal to that for w^{bl} for half a degree drop in temperature; in the brown series some of the alleles fall in groups and the rise in amount in these groups is arithmetic, being an amount approximately equal to that for w^{bl} for one degree drop in temperature. Some gaps occur in both series, and it is postulated that these missing members have not yet been recognised.

4. Possible pseudoallelism at the white locus is discussed, as well as the possible type of mutation at this locus, and the interrelations of its function with the action of other loci in the two chains of pigment production.

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ADDENDUM

After going to press a new white allele came to hand, with the wine or coral type of eye-colour. Its red pigment amounts to 0·0407 (thus filling the gap in the series between w^{sat} and w^{co}), while an amount of brown pigment of 0·0370 places it in a group with w^{w}.

THE EYE-PIGMENTARY SYSTEM OF *DROSOPHILA*

VIII. SERIES OF MULTIPLE ALLELES

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THE study of multiple alleles at a locus is of prime importance for research on the theory of the gene—its qualitative and quantitative effects and the relationship between function and mutation. In an effort to obtain data of this nature in regard to the process of eye pigmentation in *Drosophila melanogaster* nine series of multiple alleles, that is, at the loci of scarlet, vermilion, cinnabar, brown, pink, carnation, garnet, raspberry and prune, were investigated for the histology of the four eye-pigment regions and the spectrophotometric properties of the red and brown pigments. These methods have been fully described (Nolte, 1952, 1954). These multiple alleles consisted of the following:—scarlet (*st*) and scarlet-spotted (st^{sp}); vermilion (*v*) and vermilion^{36f} (v^{36f}); cinnabar (*cn*) and cinnabar^{35k} (cn^{35k}); brown (*bw*), brown^{2b} (bw^{2b}), brown-dominant (bw^{D}) and brown^{4} (bw^{4}); pink (*p*) and pink-peach (p^{p}); carnation (*car*) and carnation^{2} (car^{2}); garnet (*g*), garnet^{2} (g^{2}), garnet^{3} (g^{3}) and garnet^{4} (g^{4}); raspberry (*ras*), raspberry^{2} (ras^{2}) and raspberry^{3} (ras^{3}); prune (*pn*) and prune^{2} (pn^{2}).

2. OBSERVATIONS

(i) Phenotype

The macroscopic phenotypes of some of these alleles, in the stocks maintained in this laboratory, differ somewhat from the descriptions as given by Bridges and Brehme (1944), as will duly be noted. The eye-colour of st^{sp} is very like that of the wild-type, with perhaps a pitted effect, *i.e.*, small dark spots, but is not speckled; the combination st^{sp}/st is bright red and not speckled. The eye colour of v^{36f} is a softer bright red than that of *v*, and of cn^{35k} is a darker red than that of *cn*; the combination cn^{35k}/cn is bright red. The alleles *bw*, bw^{2b} and bw^{D} have a translucent garnet eye-colour, with that of bw^{2b} more reddish than that of *bw* and that of bw^{D} more purplish or darker; the allele bw^{4} is wild-type in phenotype, and in the combination bw^{4}/bw the colour is not a darker brown but resembles that of the wild-type although it looks somewhat darker than normal. The allele p^{p} has an eye colour, after ageing, which is more a brownish-ruby than the dull purplish-ruby of *p*. The two alleles *car* and car^{2} have very similar eye colours, of the dark ruby or garnet type. In the garnet series the alleles *g* and g^{3} are very alike, having a deep ruby or garnet eye colour, while g^{2} and g^{4} are alike in having a more translucent ruby eye colour, with the latter perhaps slightly the lighter.

The eyes of *ras* are darker than the dark translucent ruby or garnet of *ras*^{2}, while for *ras*^{3} the eye colour of the female is wild type and of the male a translucent maroon darkening to wild type within a day or two after emergence. The allele *pn*^{2} has ruby eyes, lighter than the dark brown-purplish garnet of *pn*.

(ii) Histology

The histological pictures of each series of multiple alleles will be compared with that of the alleles already described in previous studies. Sections of the eyes of *st*^{sp} resemble those of *st* excepting that the larger-sized granules of *st* were not observed (Nolte, 1950); no definite evidence of variegation (or absence of pigment in parts of the ommatidia) was found. Histologically the eyes of *v*^{36f} resemble those of *v*, and of *cn*^{35k} those of *cn*; it appears as if the basal pigment cells of these four mutants are smaller than they are in the wild-type. The allele *bw*^{2b} has a coloration in the four pigment regions similar to that described for *bw* (Nolte, 1950), but for *bw*^{D} the colour of all pigment granules is a darker brown, with the secondary and post-retinal pigment regions brown and not purplish-brown as in the other two alleles, while the rhabdomes seem to be coloured yellow; in all three alleles some large granules appear in the primary pigment cells. The allele *bw*^{4} resembles wild-type in eye histology, and so does the combination *bw*^{4}/*bw*. Whereas all four pigment regions in the mutant *p* are brown in colour, in the allele *p*^{p}, although the general colour tone is lighter, the basal and post-retinal regions look darker than the distal regions; in both alleles some granules larger than normal occur in the secondary pigment cells. The alleles *car* and *car*^{2} are similar in the coloration of the various pigment regions and both possess the very small basal pigment cell-masses (Nolte, 1950). In the garnet series the allele *g* resembles *g*^{3} (Nolte, 1950) in its eye histology, although in the primary cells *g*^{3} has larger and darker brown granules while in *g* this region resembles the other three regions in colour tone and granule size. The alleles *g*^{2} and *g*^{4} present a picture similar to that of *g*^{3} excepting that they are lighter in colour. In the raspberry series the allele *ras* resembles *ras*^{2} (Nolte, 1950), although in *ras* the brown granules of the primary pigment cells seem more yellowish in aggregate and this colour extends into the distal parts of the secondary cells. In *ras* the secondary pigment cells are less disrupted than in *ras*^{2}, the rounded masses being much less in number. More often these cells (or their pigmented granules) show a stringy appearance in *ras*, this being correlated with the more regular orientation of the basal pigment cells. The allele *ras*^{3} has, in both sexes, eyes which appear to be typically wild-type in histological structure and colour tone. The allele *pn*^{2} resembles *pn* in the disrupted appearance of the secondary pigment cell region (Nolte, 1955) and the irregularity in number and orientation of the basal pigment cells, but the colour is a much lighter brown.

(iii) Photometric analysis

The red and brown pigments extracted from the eyes of all these mutants by the AEA and AMA solvents respectively are typical of those of the wild-type, with the exception of the AEA extract of *ras* and *ras*^{2}. In these alleles light absorption in the ultra-violet range is modified as is seen in fig. 1, in which the spectrophotometric curves of the AEA extract are compared with that of *pn* which is of the normal type. The data for the quantitative determinations of the red and brown pigments were obtained for 10-12 cultures of each strain and are given in the table, the measurements being that of extinction (E) at 480 mμ for the red pigment and at 444 mμ for the brown pigment, for concentrations of 10 heads per 1 c.c. solvent.

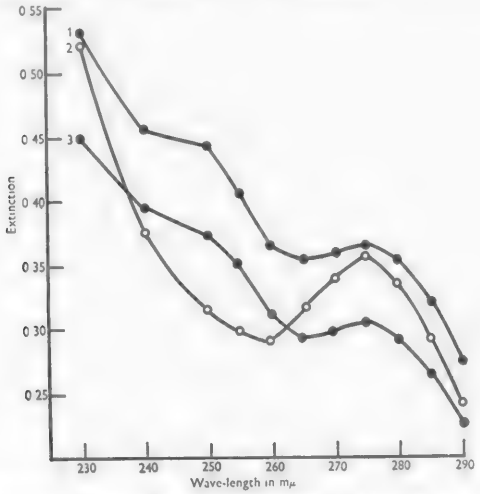

FIG. 1.—Light-absorption curves of the AEA extracts of the heads of the mutants prune, raspberry and raspberry^2. 1, *ras*; 2, *pn*; 3, *ras*^{2}.

3. DISCUSSION

The study of multiple alles affecting eye-colour in *Drosophila* yields data relevant to the study of genic action and gene mutation, additional to that obtained in the study of the multiple alleles of the white locus (Nolte, 1959).

(1) Excepting in the raspberry alleles, all the alleles in the different series produce the normal type of red and brown pigments. In a previous study (Nolte, 1952) the spectrophotometric curve was determined for the AEA extract of *ras*^{2} eyes, but only in the region of visible light in which region the curve seems typical of the red pigment. However, correlated with the exceptional reddish-violet colour of the granule masses in the secondary, basal and post-retinal regions of both alleles (Nolte, 1950) there appears a modification in ultra-violet

absorption with a valley at 265 mμ instead of at 260 mμ and a peak at the usual 275 mμ. The rise in absorption at this peak is flattened in comparison with other mutants, and the drop in absorption for the range 240-260 mμ is different to that of the normal red pigment curve. To some extent these histological and spectrophotometric data indicate the presence of a modified constituent in the red chromophore group. In the allele *ras*^{3} no such modification is evident.

TABLE

Relative amounts of red and brown pigments in the eyes of the series of multiple alleles

Strain	Red pigment	Brown pigment
Canton-S+	0·8993±0·0052	0·0866±0·0007
st	0·8910±0·0043	0·0049±0·0002
st^{sp}	0·9339±0·0066	0·0240±0·0016
v	0·8574±0·0034	0·0045±0·0001
v^{36f}	0·8641±0·0055	0·0030±0·0003
cn	0·8648±0·0019	0·0044±0·0002
cn^{35k}	0·9054±0·0114	0·0041±0·0002
bw	0·0110	0·0750±0·0006
bw^{2b}	0·0130	0·0688±0·0013
bw^{D}	0·0120	0·0906±0·0005
bw^{4}	0·8976±0·0091	0·0837±0·0009
p	0·3608±0·0057	0·0284±0·0005
p^{p}	0·0834±0·0013	0·0133±0·0002
car	0·2999±0·0044	0·0403±0·0011
car^{2}	0·1454±0·0055	0·0419±0·0014
g	0·3380±0·0058	0·0485±0·0013
g^{2}	0·1384±0·0044	0·0274±0·0004
g^{3}	0·1900±0·0033	0·0409±0·0012
g^{4}	0·2037±0·0036	0·0200±0·0003
ras	0·2213±0·0045	0·0955±0·0014
ras^{2}	0·1361±0·0017	0·0891±0·0007
ras^{3}	0·8941±0·0116	0·0906±0·0032
ras/*ras*^{3}	0·1830	0·0850
ras^{3}/*ras*^{3}	0·7200	0·0930
pn	0·2318±0·0012	0·0960±0·0008
pn^{2}	0·1654±0·0027	0·0989±0·0008

(2) The quantitative data of the table include results obtained in previous investigations for various members of the different series of alleles (Nolte, 1954, 1955); for purposes of comparison the pigment content of the Canton-S wild-type strain is included. Two facts are to be kept in mind for the estimation of the significance of quantitative differences. First, the various strains are not equal in size, and size differences will naturally affect the pigment content differences. Secondly, the strains are not isogenic for any series of genes which may affect the total amounts of pigment. Taking size differences into consideration the following are significant quantitative pigment differences.

The allele *st*^{sp} produces somewhat more red pigment and about five times more AMA soluble material than does *st*. In the brown alleles there is a significant increase in brown pigment in the series

$bw^{2b} \rightarrow bw \rightarrow bw^4 \rightarrow bw^D$. The rate of increase in the series is arithmetic and by nearly equal amounts for each step; bw^4, however, has an amount of red pigment equal to that of the wild-type. The allele p produces about four times the amount of red pigment and about twice the amount of brown pigment that are produced by p^p. As already shown in a previous study (Nolte, 1952) *car* compared with car^2 produces about twice the amount of red pigment but an equal amount of brown pigment.

In the garnet series quantitative relations occur which are similar to those determined for the alleles of the white locus (Nolte, 1959). For this series it is likewise postulated that not all members have been recognised. If an allele of garnet (g^x) be assumed to contain the relative amounts of about 0·25 of red pigment and 0·034 of brown pigment, then there is a geometric rise in red pigment content in the series $g^2 \rightarrow g^3$, $g^4 \rightarrow g^x \rightarrow g$, the amount of increase being by a factor of about 1·35 for each step. The brown pigment would then show an arithmetic increase in content in the series $g^4 \rightarrow g^2 \rightarrow g^x \rightarrow g^3 \rightarrow g$, the amount of increase for each step being about one-third of the amount present in the lowest member. These increases are equivalent to the rates of increase of the two pigments in the allele w^{bl} for a drop in temperature of 1° C. (Nolte, 1959). The eye colour of this presumed allele would probably resemble that of the mutant *car* (Nolte, 1950).

There is a lesser degree of disarrangement of the secondary pigment region in *ras* than in ras^2 but a greater amount of pigment in the eyes. In *ras* the brown pigment occurs in a relatively greater amount than in the wild-type. The allele ras^3 does not appear to affect the pigment content in the female, but taking into account the differences in eye size between the sexes, it seems from the data of the few determinations made for males that the eyes of the latter have a more or less equivalent amount of brown pigment but about one-half the amount of red pigment found in the eyes of females. In combinations between these alleles there exists an additive effect for the red pigment, but not for the brown pigment.

In the prune series the allele *pn* produces about one-half more red pigment than pn^2 but the amounts of brown pigment are about equal; both alleles produce significantly more brown pigment than does the wild-type.

(3) The known and presumed functions of the different loci to which these multiple alleles belong have been discussed in previous studies (Nolte, 1952, 1954, 1955). The scarlet locus has been postulated to act at the level of formation of brown chromophore from chromogen and substrate, this process being blocked by *st*. The allele st^{sp}, therefore, blocks this reaction to a lesser extent; the fact that this allele produces more red pigment than does the wild type may be the result of more substrate being available for the red pigment chain of reactions. The vermilion and cinnabar loci act during the production of brown

chromogen from tryptophane. The differences in phenotype and pigment content between *v* and v^{36f} and between *cn* and cn^{35k} are so small that it seems possible that these are not pairs of alleles but the same allele in each case in a different genotypic background. If the function of the brown locus is assumed to be the combination of red chromogen with substrate to form the red chromophore group, the alleles at this locus evidently block this reaction, excepting in the case of bw^4. However, in a single determination the combination bw^4/bw was found to produce the relative amounts of 0·7102 of red pigment and 0·1050 of brown pigment, indicating that bw^4 has an action at the level of the threshold value. The production of the brown chromophore group is also affected by the alleles bw^4, *bw* and bw^{2b}, the decrease in content being in an arithmetic series in that order. This secondary effect of the mutants at this locus may be the result of a disturbance of the balance of reactions leading to the production of chromophore groups in the two chains.

The functions of the loci of pink, carnation and garnet have been assumed to be the directing of the specificities of enzymes utilised during the histolytic and synthetic processes of protein metabolism during metamorphosis. A product formed during these reactions is probably utilised in the chain leading to the common substrate for the differentiating action of the white locus. The two alleles *p* and p^p are hypomorphic in their action, affecting the content of both pigments, p^p slowing down the production of the red pigment to a relatively greater extent than that of the brown, as compared with *p*. A secondary effect of mutation at this locus is a disturbance of normal pigment granule formation. In the carnation and garnet loci we again find that the multiple alleles affect the production of the red pigment to a greater extent than that of the brown. In garnet the geometric rate of increase of red pigment in the alleles and the arithmetic rate of increase of the brown pigment are very similar to those found for the alleles of the white locus (Nolte, 1959). The latter locus acts at the end of the reaction chain on a substance provided, amongst others, by the action of the garnet and carnation loci; at both loci the alleles show qualitative differences, when arranged in series, in regard to the quantitative effect on the two pigments.

The function of the locus of raspberry has been suggested to be that of synthesis of cytoplasmic proteins for pigment cell differentiation; the mutant alleles cause a decrease in number and a disturbance in the orientation of the basal pigment cells and this results in a disorganization of the secondary pigment region, more so in ras^2 than *ras*. Correlated with this disruption is the decrease in red pigment content and the slight modification of part of the red chromophore group, a pointer to the possibility that a substance (protein) used both in cell and chromophore production is modified. The significant increase in brown pigment content in *ras* as compared with the wild-type might be a secondary effect of the disturbed balance in the

processes leading to pigment deposition. In the case of *ras*^{3} the mutation seems to have reduced the efficiency of the locus to the level of the threshold of activity. The locus of prune has also been shown to

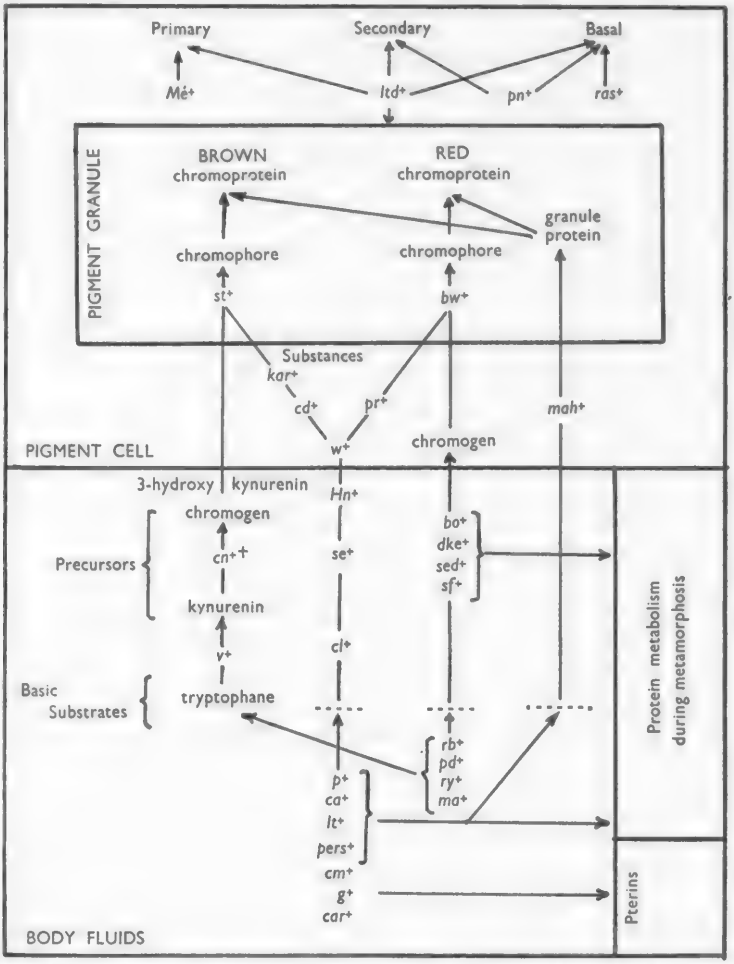

FIG. 2.—Diagrammatic representation of the known and postulated functions of the main eye-colour genes in *D. melanogaster*.

affect the differentiation of the pigment cell regions. The allele *pn*^{2} shows a greater amount of disorganization of the secondary pigment cell region than does the allele *pn*, and a correlated greater decrease in red pigment content but a relatively higher brown pigment content; the amount of brown pigment is significantly greater in both

alleles than in wild-type. These quantitative effects may result secondarily from disturbances in the methods of deposition of the two pigments.

The discussion on the known and presumed functions of the eye-colour genes that have been investigated in this study and in others (Nolte, 1952, 1954, 1955) may be clarified by a diagrammatic representation (fig. 2).

4. SUMMARY

The seventh group of eye-colour mutants of *Drosophila melanogaster* to be studied histologically and spectrophotometrically comprises the series of multiple alleles at the loci of scarlet, vermilion, cinnabar, brown, pink, carnation, garnet, raspberry and prune.

1. Histologically the multiple alleles of a series produce similar specific effects, *e.g.* in the general colour of the four pigment regions, in the type of granules produced and in the orientation of the pigment cells. Thus *p* and p^p produce some granules larger than normal in the secondary pigment cells; *car* and car^2 have very small basal pigment masses; the alleles g^2, g^3 and g^4 appear to have brown pigment only in the primary cells; the alleles *ras* and ras^2 show a decrease in the number of basal cells and a clumping of the pigment masses in the secondary cells; in *pn* and pn^2 a similar picture of disorganization of the secondary cells appears.

2. All the mutants produce the normal types of red and brown eye pigments excepting *ras* and ras^2 for which the red pigment extracts shows a modified photometric curve in the ultra-violet region, indicating a modification in the red chromophore group.

3. The quantitative relations of the two pigments vary for the different series of alleles. In the brown series the brown pigment shows an arithmetic increase in the four alleles; in three alleles the red pigment is absent but in bw^4 the normal amount is present. The two alleles in each of the pink and carnation series show a greater difference in the red than in the brown pigment content. In the garnet series the existence of a fifth member (g^x) is postulated and if this is so the red pigment shows a geometric rate of increase while the brown pigment content rises arithmetically for a different order of the alleles. In the raspberry and prune series the disarrangement in the pigment cell regions is correlated with a decrease in the red pigment content but an increase in brown pigment. In ras^3 the activity of the locus has apparently been lowered to the threshold value (lower in the hemizygous male).

4. The known and presumed functions of these loci are discussed.

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STUDIES OF LINKAGE IN POPULATIONS

III. AN ASSOCIATION OF LINKED INVERSIONS IN *DROSOPHILA GUARAMUNU*

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ONE of us (Levitan 1954*a*, 1954*b*, 1955, 1957, 1958*b*) has recently found that linked gene arrangements of *D. robusta* exist in non-random associations in two woods near Blacksburg, Virginia. Conclusive demonstration of the associations involved laborious crosses of wild-caught adults to laboratory strains. Using a basically similar method to analyse several wild strains Brncic (1953) noted that two widespread " independent " inversions *E* and *H* on the fourth chromosome of *Drosophila guaramunu* are " almost always associated in the same chromosome ". In the data of da Cunha, Brncic and Salzano (1953) and Salzano (1955) studying populations of *D. guaramunu* from several Brazilian localities these two arrangements were heterozygous with almost identical frequencies in each place. The present paper, using the raw data of Salzano (1955), and some unpublished data of that author, will show that the association of these inversions is a most remarkable one which can be conclusively demonstrated even in larval (" egg sample ") data.

Materials and methods, localities studied, etc., are given in the cited papers of Brncic, da Cunha *et al.*, and Salzano. Briefly, the data by Salzano discussed here were derived from study of the salivary chromosome of one female larva from each inseminated wild-caught female (so-called " egg " samples). For the *H* inversion the results were recorded as to heterozygosity (*Hh*) or homozygosity for arrangement, the two types of homozygotes, *HH* and *hh*, not being distinguishable; for *E*, however, all three karyotypes, *EE*, *Ee* and *ee* were distinguished for all collections after September 1952. The few data of Salzano obtained prior to September 1952 will be ignored.

Both *E* and *H* are simple (two break) paracentric inversions with respect to Standard (Brncic, 1953). Both are moderate in size, *E* extending about half again the length of *H*. The euchromatin in the uninverted section between them, very nicely shown in fig. 3 of Brncic (1953), consists of at least thirty eight bands, about fifty if double bands are counted as two bands each. (A comparison of the figure cited with Brncic's map of the salivary chromosome indicates that, contrary to the text, the distance between the inversions includes sections 70, 71, and 72; this interpretation has been confirmed by the observations of these inversions by the junior author.)

The data for the four localities where all *E* karyotypes were determined are shown in table 1. Not a single instance of *ee* with *Hh* was recorded in the 1151 larvæ examined. Since *ee* were rare in most of the collections this might be attributable to chance, but such is

TABLE 1

Egg sample karyotypes at the E *and* H *inversion loci on chromosome-4 of* D. guaramunu *from four Brazilian localities*

Locality and date	*N*	*EE* & *Hh*	*Ee* & *Hh*	*ee* & *Hh*	All *EE*	All *Ee*	All *ee*	*Ee* with *HH* or *hh*
Bexiga								
Feb. '53	100	0	43	0	40	43	17	0
May '53	100	0	39	0	24	39	37	0
Aug. '53	100	2	40	0	17	40	43	0
Nov. '53	100	4	16	0	82	16	2	0
All Bexiga	400	6	138	0	163	138	99	0
Emboaba								
Jan. '53	88	1	9	0	68	9	11	0
July '53	14	0	1	0	2	1	11	0
Oct. '53	100	2	16	0	76	16	8	0
All Emboaba	202	3	26	0	146	26	30	0
Feliz								
Dec. '52	100	1	20	0	78	20	2	0
Feb. '53	100	1	17	0	83	17	0	0
June '53	100	3	22	0	73	22	5	0
Aug.-Sept. '53	80	0	14	0	61	14	5	0
Dec. '53	100	6	24	0	67	24	9	0
All Feliz	480	11	97	0	362	97	21	0
Ponta Grossa								
Oct. '52	19	0	5	0	2	5	12	0
July-Aug. '53	50	0	16	0	2	16	32	0
All Ponta Grossa	69	0	21	0	4	21	44	0

certainly not the case at Ponta Grossa and in three of the four Bexiga collections. At Feliz, furthermore, the deficiency of *ee* with *Hh* is significant in the total sample: *ee* being 4·4 per cent. (21/480) of the total, one would expect by chance 4·4 per cent., or 4·8, of the 108 *Hh* individuals to be *ee*; the deviation from expected has χ^2 of 5·0 for one d.f. (P about ·025) without Yates' correction, χ^2 of 4·0 ($P \leqslant \cdot 05$) with Yates' correction. Likewise at Emboaba, χ^2 is 4·8 (P about ·04) without Yates' correction, 3·7 (P about ·06) with Yates' correction. For a composite of all samples with few *ee* or few *Hh* (the Spring Bexiga and all the Emboaba and Feliz data), the deficiency of *ee* with *Hh* from expected (17·4) is highly significant (as it is in the three Bexiga and the Ponta Grossa samples where *ee* was common).

Hh larvæ of karyotype *EE* do occur at all three localities, but these also are rarer than expected. The deficiences are highly significant in every large sample ($N \geq 80$) except the winter Bexiga one (where χ^2 is 4·46 with P about ·04, *i.e.* barely significant).

Turning to the *Ee* larvæ, one can determine how often they were found with an homozygous "*H*" karyotype (*HH* or *hh*) by subtracting the total number of *Ee* (column 5) from the number of "*Ee* with *Hh*" (column 2). The results are shown in the extreme right-hand column. Out of 1151 larvæ not a single instance of *Ee* that was not also *Hh* was found. The deficiency of "*Ee* with *HH* or *hh*" from the number expected by chance (based on the number of *Hh* and *hh* present) is highly significant in every sample of fifty or more larvæ, even the summer Emboaba one with only nine *Ee*.

To sum up, of the 1151 larvæ recorded for the linked inversions *E* and *H*, only 20 (1·7 per cent.) were heterozygous for one arrangement and homozygous for another (*EEHh*, *eeHh*, *EeHH*, *Eehh*), the other 1131 (98·3 per cent.) being either doubly homozygous (*EEHH*, *EEhh*, *eeHH*, and *eehh*) or doubly heterozygous (*EeHh*). The double homozygotes total 849 (73·8 per cent.), the double heterozygotes 282 (24·5 per cent.). No imaginable karyotype frequencies at the two inversion loci could produce such a distribution of zygotic combinations by chance. Clearly a non-random association has been demonstrated here despite the handicaps that only larval ("egg sample") data are available and not all karyotypes could be distinguished at one of the loci.

Two explanations of these data seem possible, one at the chromosome or "gametic" level, the other at the zygotic level. At the gametic level, assume one of the linkages to be most primitive, say *EH*. Inversion of, say, the "*E*" segment produced *eH* chromosomes, then inversion of the "*H*" segment on either *EH* or *eH* produced, say, *Eh* (assuming the inversion occurs on *EH*). Confining our attention to the larger samples at Bexiga, Emboaba and Feliz, it may be noted that *EE* has a minimum frequency of 17 per cent., and occurs almost exclusively with homozygotes for the "*H*" locus (in linkage notation, *EH*/*EH* or *Eh*/*Eh*); this means that in these populations either *EH* or *Eh*, *but not both*, must be common. If both were *common*, *EE* with *Hh* (*EH*/*Eh*) would not be such a deficient combination; and if both were *rare*, homozygous *EE* would be rare, the frequency of *EE* being at least the square of the frequency of *E* in each sample. (Note that even where highly significant deviations from the Hardy-Weinberg Law occur, in the two large Emboaba and the summer Feliz samples, they involve an *excess* of *EE*.) Crossing-over in *Eh*/*eH* females would produce some *eh* chromosomes. Whether these have become common or remained rare depends on which of the *E* linkages, *EH* or *Eh*, became the common one. If *Eh* became common, then *eh* must have remained rare, otherwise *Eehh* (*Eh*/*eh* in linkage form) would be more frequent; *eH*, on the other hand, would then need to have attained

a fairly high frequency, for frequent *Eh* means that the common double heterozygotes (*Ee* with *Hh*) are almost exclusively of linkage type *Eh*/*eH*. The argument would be reversed if *EH* became the common "*E*" linkage: *eH* must then have remained rare (hence the rarity of *EH*/*eh*) and *eh* common (to account for the frequency of "*Ee* with *Hh*", which are almost exclusively *EH*/*eh* when *eH* and *Eh* are rare). In any case, it can be seen that either the two coupling or *cis* linkages (*EH* and *eh*) must be common, the two repulsion (*trans*) linkages (*Eh* and *eH*) rare, or vice versa, to account for the data.

Brncic (1953) has suggested that the probable phylogeny in these populations is (re-written here in linkage form):

$$eh \longleftrightarrow Eh \longleftrightarrow EH,$$

the two end members being the most common in these populations. On this scheme linkage *eH*, if it exists, would have arisen by crossing-over. As Brncic points out, other possible schemes, albeit less probable, are consistent with the data, as, for example:

$$eh \longleftrightarrow eH \longleftrightarrow EH \longleftrightarrow Eh$$

or,

$$Eh \longleftrightarrow eh \longleftrightarrow eH \longleftrightarrow EH,$$

in each of which either of the end members arose by crossing-over. These alternative schemes assume *eH* is a comparatively old arrangement which has proven adaptively weak in these populations. The final conclusion in the previous paragraph is valid, however, no matter what the phylogeny (or notation) of the arrangements is. It should also be noted that on any scheme one of the two common linkages must have become more frequent, via natural selection, than at least one linkage of earlier origin. For example, if *EH* and *eh* are now the most common combinations, of which *EH*, say, is the more primitive, *eh* has become more common than *Eh* and *eH*, at least one of which antedate it.

D. robusta also has an association characterised by excess of the two coupling linkages and deficiency of the two repulsion linkages (Levitan, 1954*b*, 1957 and 1958*b*). The deviations from randomness are small, albeit significant, and were not suspected from the egg sample data. The *XL*-2 . *XR*-2 association of *D. robusta* (Carson and Stalker, 1949; Carson, 1958; Levitan, 1958*a*, *b*), on the other hand, does resemble this one in that it may be inferred, though not as conclusively, from egg sample data. The probable phylogeny is:

$$XL\,.\,XR \longleftrightarrow XL\,.\,XR\text{-}2 \longleftrightarrow XL\text{-}2\,.\,XR\text{-}2$$

The combination *XL*-2 . *XR* (analogous to *eH* here), probably produced by crossing over, is extremely rare or absent in natural populations. Unlike the *D. guaramunu* association, the two most common linkages are probably the oldest, one a "coupling", the other a "repulsion" linkage, but *XL*-2 . *XR*-2 is more frequent than at least

one of its two probable predecessors in two recorded samples of more than 20 chromosomes (samples 16 and 18 in table 4 of Carson, 1958) and in several smaller ones (*op. cit.*). Both the *D. guaramunu* and *D. robusta* associations demonstrate, then, instances when a phylogenetically more recent linkage combination has increased in frequency by natural selection, its greater adaptive value overcoming the tendency of crossing-over to break it down.

This conclusion seems warranted even though the existence of linkage *eH* in these populations is uncertain. It may be argued that inasmuch as the space between inversions *E* and *H* is small (presumably 10-15 per cent. of the euchromatin of chromosome-4), possibly crossing-over between the two has never occurred. This seems highly unlikely, however, since crossing-over in even smaller regions have been recorded, for example, in the well-known distal end of the X-chromosome of *D. melanogaster*, not to speak of the crossing-over in pseudo-alleles. Wasserman and Wilson (1957) report the existence of all four combinations of independent inversions $2s^2$ and $2y^2$ and their Standard counterparts in a Mexican species of the *repleta* group, *D. nigricruria*, indicating that crossing-over has occurred at least once in the uninverted region between the inversions. According to Wasserman and Wilson (*op. cit.*) this region, between sections E3c and E4a of chromosome-2, contains " approximately ten bands "; judging from the *D. repleta* map of Wharton (1942), one would assume their statement includes light- as well as dark-staining bands. In any case, the region is considerably smaller than the one between *E* and *H* of *D. guaramunu*.

An alternative hypothesis is that the non-random condition of the linkages is a by-product of competition at the zygotic level, the double homozygote with, say, coupling combinations being adaptively superior to the homozygotes for the reverse (repulsion) combinations and to the individuals homozygous for one arrangement, heterozygous for the other. The double heterozygotes would be almost exclusively of the same nature as the dominant homozygotes (opposite coupling linkages in the example). This seems a more difficult system to evolve than the one envisaged by the gametic level hypothesis, but our data do not enable us to distinguish between the two hypotheses. A re-study of the populations distinguishing between the homozygotes *HH* and *hh* may determine which is correct. The most significant point is that under either hypothesis some adaptive interaction between independent inversions must be postulated to explain the non-random association.

SUMMARY

Drosophila populations in four Brazilian localities contain an extreme non-random association of two " independent " inversions of the fourth chromosome. Individuals homozygous for one arrangement and heterozygous for the other are very rare in the populations,

over 98 per cent. of the egg sample larvæ being either heterozygous for both inversions or homozygous at both inversion loci.

The results are probably attributable to differential selection for chromosomes with certain combinations of the arrangements, the two coupling (*cis*) combinations probably being favoured, the two repulsion (*trans*) combinations selected against.

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STUDIES IN WILD SPECIES OF COTTON

I. VARIATION WITHIN *GOSSYPIUM ANOMALUM*

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1. INTRODUCTION

Gossypium anomalum Wawra and Peyr is a wild diploid cotton indigenous to the African continent. It lacks spinnable lint having only a short khaki fuzz appressed to the seed. This species and *G. somalense* have been collected in natural vegetation in the Sudan, and their distribution in that country has been described by Knight (1949). A botanical description of *G. anomalum* was published by Hutchinson, Silow and Stephens (1947).

One accession only of *G. anomalum* has been extensively studied. This work was carried out by Silow (1941) who investigated the comparative genetics of *G. anomalum* and the Asiatic cottons. The type he used represents the most widely known form of the species and is maintained under the number WB1-1. At the present time 15 accessions of *anomalum* are grown at Shambat, near Khartoum, in the northern Sudan. These represent the following areas: 1 from W. Africa (WB1-1), 10 from different districts of the Sudan and 4 from southern Africa.

The 15 types are grown perennially in the collection of wild species. The most striking feature of these representatives of *anomalum* from widely separated areas is their remarkably uniform appearance. Small differences are discernible in habit; there is some variation in boll shape; the southern types flower somewhat later than the northern accessions and 3 of the 4 southern types have a magenta flower colour, the northern all being cream. Only one glabrescent *anomalum* has been found and this was collected in the south. One accession from the Sudan was found to possess a distinct leaf shape.

Because the variation within *anomalum* described above has so far not been reported, this study was carried out. The genetics of flower colour, leaf shape and hairiness will be discussed here.

2. MATERIALS AND METHODS

The strains of *G. anomalum* used in this study were:

WB1-1: Densely hairy on all parts of the leaf and stem; pale cream flower sometimes tinged with anthocyanin; ¾ cut leaves usually 5-lobed. Collected in W. Africa.

WB1-5: Differs from WB1-1 only in that the leaves are about ½ cut into 3 broad lobes. Collected in the Sudan.

WB1-14: Magenta-coloured flower; glabrescent leaves and plant body. Leaf shape similar to WB1-1. Collected in S.W. Africa.

WB1-5 and WB1-14 were crossed with WB1-1 and F_2 and F_3 families were raised. The results will be given below.

The seeds of *anomalum* possess a very hard testa and because of this seeds germinate very erratically under ordinary conditions of cultivation. All seeds were therefore chipped, germinated on damp filter paper and raised as seedlings in a greenhouse for later planting in the field.

Cotton flowers are selfed at Shambat by clipping a small endrin-impregnated paper bag over the unopened flower. A poor set was obtained with *anomalum* treated in this way and the F_2 families were small in consequence. This difficulty was overcome by removal of the bag at the time when the flower had opened for mechanical application of self pollen to the stigma with subsequent replacement of the bag. When this was done a satisfactory boll setting was obtained.

3. RESULTS

(i) Flower colour

Flower colour was graded by eye estimation and five grades were used; a description follows:

Grade	Description	Parents and Hybrids
1	Pale cream	WB1-1
2	Pale cream tinged pink	range
3	Weak magenta	WB1-14
4	Moderate magenta	range
5	Strong magenta	Hybrid segregates only

Nine F_1 plants were grown from the cross WB1-1 (cream) × WB1-14 (magenta). Although a note was made that this F_1 was a light magenta colour no grade was recorded since at that time the cross was intended solely for a study of hairiness. From these nine plants F_2s were grown.

It was decided in 1956 that these families should be used for a study of corolla colour inheritance. At the end of the season it was realised that the method of grading colour was unsatisfactory and that the assessment of tones in the bright glare of the sun could be subject to considerable error.

One entire F_2 family G233/56 was individually selfed and the resultant F_3s raised in 1957. Colour assessment was made on flowers brought into the laboratory and examined at a well-lit window out of the glare of the sun. The parent types were always available for comparison. It was found that by using this method flowers could be placed with complete confidence into one or another of the grades 1 to 5. The results are set out in table 1.

Differences in flower colour grade can be due to both genetic and environmental influences. Silow (1941) recorded variability of anthocyanin expression within plants and explained this as being due to differences in exposure of flower buds to the sun. The writer has

repeatedly observed flowers of differing grades upon the same plant. This being the case, family grading based upon one flower per plant can obviously be subject to error, but this error has to be accepted since replication of flower colour assessment was not a practicable measure in view of the amount of work involved. Since this type of error can equally well occur in all families, differences in mean grade are more likely to be due to genetic causes.

A complete understanding of the nature of corolla colour inheritance within *anomalum* would require considerably more extensive study

TABLE 1

F_3 families from the cross WB1-1 × WB1-14

G Family 1957	Colour Grades					Total	Mean Grade
	1	2	3	4	5		
WB1-1	...	...	...	...	...	...	1·5
WB1-14	...	7	23	19	...	*49*	3·2
G222/57	33	2	2	...	...	*37*	1·2
220	36	22	...	...	...	*58*	1·4
228	24	7	[illegible]	...	...	*34*	1·4
217	54	46	3	...	...	*103*	1·5
219	23	22	1	...	...	*46*	1·5
225	47	73	10	...	...	*130*	1·7
223	18	17	10	1	1	*47*	1·9
227	37	47	26	1	...	*111*	1·9
221	30	39	21	3	...	*93*	2·0
218	7	30	21	19	...	*77*	2·7
224	3	[illegible]	46	22	1	*80*	3·1
226	...	7	20	62	2	*91*	3·6

than it has been possible to give it here. However, the data presented shows more variation than would be expected if the control of colour was monogenic. This clearly points to polygenic influence on the intensity of pigmentation.

(ii) Leaf shape

The leaf-shape gene normally encountered in *anomalum* was given the symbol L^4 by Silow (1939) who found that it was a member of a multiple allelic series for leaf shape in the Asiatic cottons. He used two indices to record leaf shape, they were :—

$$\text{Index C} = \frac{\text{Sinus Length}}{\text{Leaf Length}}$$

$$\text{Index D} = \frac{\text{Lobe Width}}{\text{Leaf Length}}$$

These indices are reciprocals of those used in earlier studies on the cotton leaf made by Hutchinson (1934) and are used in the work reported here. Indices are × 100 eliminating decimals.

L^A is the leaf-shape gene of WB1-1, a $\frac{3}{4}$ cut palmatifid leaf of 5 lobes (fig. 1*a*). WB1-5 has a leaf $\frac{1}{3}$ palmately cut and is 3-lobed (fig. 1*b*). In 1956 nine plants from a bulk cross between WB1-1 and WB1-5 were grown and selfed. The F_2 families were grown in the season 1956/57. Of these only four segregated for leaf shape, the remainder being homozygous for the *L*^{A} gene. It was realised later that the WB1-5 stock which is grown perennially at Shambat was not pure for the leaf-shape allele under study and that some plants heterozygous for this gene and *L*^{A} were crossed to WB1-1 (*L*^{A}*L*^{A}) to produce the F_1 which was thus mixed and hence gave the non-segregating families in F_2. The families which did segregate gave 20 to 6, 16 to 4, 5 to 5 and 4 to 5 palmatified *L*^{A} types to palmate types. The two largest families suggest monofactorial segregation. In view of the small F_2 family numbers it was necessary to grow an

FIG. 1*a*.—Palmatifid.

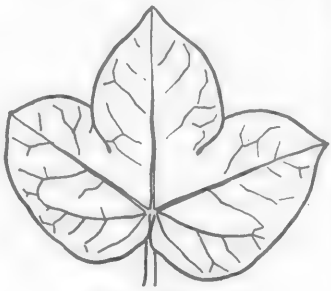

FIG. 1*b*.—Palmate.

adequate number of F_3s. One family G243/56 (giving the ratio 20 to 6 mentioned above) was entirely individually selfed with the exception of two rather weak plants, so leaving the family effectively as 19 to 5. The resulting F_3 families are set out in detail in table 2. Without exception the seven families segregating for the shape genes show excellent approximations to 3 : 1 expectations, thus confirming the indication given in the F_2 families that a single locus is involved.

It is of interest to note here that the family G243/56 with an observed 20 dominants to 6 recessives, which is an excellent fit to a 3 : 1, is in fact spuriously so when the F_3 data are examined. Table 2 shows that 12 of the F_2 plants must have been homozygous palmatifid, 7 heterozygous and 5 homozygous palmate allowing for the two plants which did not bear seed. This is in poor agreement with an expected 6 : 12 : 6.

The data for the seven segregating families have been combined and index C plotted against index D in table 3. It can be readily seen that the real difference between palmatifid and palmate lies in sinus length, lobe width hardly differing at all. Stephens (1944) when comparing *L*^{A} and *I* found that the difference lay in sinus length

and came to the conclusion that L^A ". . . has an extremely localised effect in the sinus region of the leaf".

The gene for palmatifid is incompletely dominant over that for palmate leaf. Table 3 shows that the palmatifid homozygote and the

TABLE 2

F_3 families from the cross WB1-1 × WB1-5

Parent genotype	G Family 1957	Observed		
		Palmatifid	Heterozygote	Palmate
Palmatifid	229	14	...	...
	234	32	...	...
	235	32	...	...
	237	31	...	...
	238	31	...	...
	239	45	...	...
	245	71	...	...
	246	69	...	...
	247	80	...	...
	248	57	...	...
	249	45	...	...
	251	41	2 *	...
Heterozygote	230		28	8
	232		65	20
	240		25	10
	242		6	2
	244		34	14
	250		53	17
	252		51	15
Palmate	231	...	...	81
	233	...	1 *	39
	236	...	...	33
	241	...	...	5
	243	...	3 *	33

* Possible outcrosses.

heterozygote merge into one another in respect of the indices but are distinctly separate from the palmate homozygote. In correlation tables for individual families this separation is much more marked, but in bulking the data for table 3 family differences blur the distinction. All heterozygotes had a higher value for index C than the homozygotes although this distinction was not marked enough for confidence in placing them in a separate class. Thus the type of dominance found here is in agreement with the field series of Hutchinson (1934), *i.e.* dominance is in the direction of deep-cut leaves.

Modifier control over lobe width has been noted in previous leaf-shape studies (Silow, 1939). Evidence of a lobe width modifier will be presented here also. If the mean values for C and D for each of the segregating families are plotted in such a way that each family

TABLE 3

Combined data on leaf shape from F_3 families G230 57, G232 57, G240 57, G242 57, G244 57, G250 57 and G252 57

Index D	Index C: 16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60	62	...	70		
84	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	
82	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
80	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	*1*
78	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	*1*	.	.	.	.	.	.	.	.	.	*1*
76	.	.	.	.	.	.	.	.	.	.	1	1	.	.	.	.	.	*1*	*1*	.	.	.	1	.	.	.	2	*3*
74	.	.	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.	*1*	*1*	.	.	1	.	.	.	.	3	*3*
72	.	.	.	.	.	.	2	3	.	2	2	1	1	.	.	*1*	2	2	*1*	*1*	.	1	1	1	.	.	11	*10*
70	.	.	3	.	.	1	1	7	.	1	1	.	*1*	.	.	*1*	*1*	*1*	*1*	3	.	.	.	.	.	.	14	*8*
68	.	.	1	2	2	4	5	6	6	1	.	.	1	*1*	.	.	*3*	*5*	*1*	1	.	.	.	.	.	.	28	*11*
66	.	.	4	4	2	4	8	6	2	1	2	.	.	*1*	*2*	*3*	*1*	*3*	*3*	1	1	.	.	.	.	.	33	*15*
64	1	3	3	3	2	7	10	6	1	1	1	.	1	.	*3*	*1*	*1*	*3*	.	3	1	.	.	.	.	.	39	*12*
62	1	5	3	4	4	5	6	2	2	1	3	.	.	.	*1*	.	*3*	*1*	.	1	1	.	.	.	.	.	36	*7*
60	.	5	9	3	1	6	4	5	2	3	1	1	.	.	.	*1*	*2*	*1*	.	2	.	.	.	.	.	1	40	*7*
58	3	2	4	5	6	2	1	1	2	.	2	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	28	*1*
56	1	2	4	1	1	1	2	1	1	1	.	.	.	.	.	.	.	.	1	1	.	.	.	.	1	.	15	*3*
54	.	1	1	1	.	2	.	3	.	.	.	.	1	.	*1*	*1*	.	.	.	.	1	.	.	.	.	.	9	*3*
52	.	.	1	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	
50	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
48	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	1	*1*
Observed	6	18	34	26	18	33	39	40	16	11	14	3	4	*1*	*2*	*7*	*8*	*13*	*19*	*11*	*12*	*5*	*3*	*2*	*2*	*1*	348	
	(Palmatifid homozygotes + heterozygotes) 262													(Palmate homozygotes) *86*														
Expected 3 : 1	261·00													87·00													348·00	

has two means, one for the dominant group and one for the recessive group and these two values joined by a line, this line then acts as a pointer to the related values and also defines the slope between them. The seven families are then seen to fall into three distinct groups (see fig. 2) and these groups are in the proportions 2 : 3 : 2. A further point emphasising the reality of this grouping is the noticeably different slope of the lines of the middle group, all of which are similar within the group. The two outside groups are similar to each other in slope.

These findings can be interpreted as being due to a modifier *M* which increases the W/L value; *mm* causes no change and *Mm* is intermediate in value between this and *MM*. In fig. 2 G230/57 and

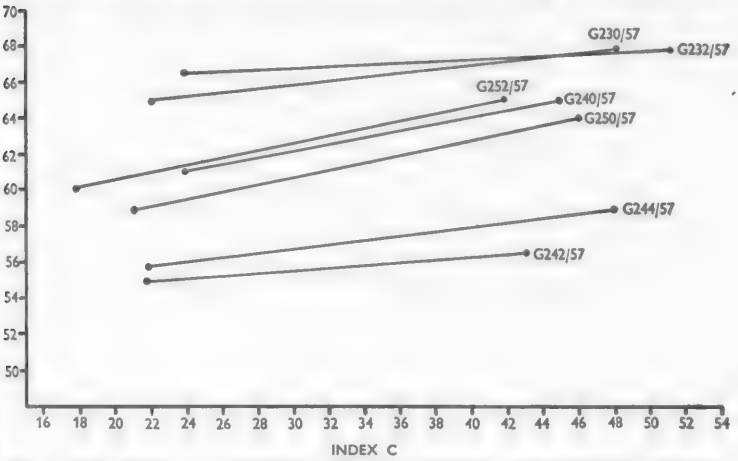

FIG. 2.—Each family is represented by two means joined by a line indicating the slope between the means. The group at the lower end of index C is Palmatifid + heterozygotes and the group at the higher end of index C is Palmate.

G232/57 then become *MM*; G240/57, G250/57 and G252/57 become *Mm*; G242/57 and G244/57 *mm*. The observed distribution of these groups of 2 : 3 : 2, while in reasonable agreement with an expected 1 : 2 : 1, cannot be taken as reliably establishing the existence of the modifier because of the small number of families. If this modifier is acting here it is of interest to note that when heterozygous (*Mm*) it acts unequally upon the palmatifid and palmate classes so giving the difference in slope observable between the middle group and the others. Silow (1939) drew attention to modifiers which acted differentially upon the three phases of a main gene. The homozygous broad leaf was made even broader, the heterozygote broader to a lesser extent and the homozygous narrow leaf hardly affected at all. The differential here appears to be in the heterozygous phase of the modifier but the effect is similar to Silow's findings in that the broad leaf is made even broader and that the narrower allele is affected to a slightly less extent.

The effect of this modifier upon the homozygous families is observable but not easily analysable since the distribution is continuous, the heterozygotic families are simpler because of the use that can be made of the angle of slope joining the dominant and recessive groups.

(iii) Hairiness

The two accessions studied may be described with respect to hair as follows:—

(*a*) WB1-1: strongly hairy with dense soft hair on both leaf surfaces, moderate length. Densely hairy on all vascular tissues with hair at two levels. Long simple single or double hairs dominant; the second level stellate hairs, eight or more arms, not more than half the length of the simple hairs and considerably less dense.

(*b*) WB1-14: distribution of hair the same as for WB1-1 but with the absence of long simple hairs from the vascular tissues. Hair very sparse on all surfaces and hence described as glabrescent.

In an analysis of the hairiness of WB1-1, it would be desirable to cross it with a fully glabrous *anomalum* but no such strain has been recorded and the glabrescent WB1-14 was therefore used. The F_1 was found to be only slightly more hairy than the glabrescent parent. Fully hairy appeared to be recessive and this was confirmed in later work.

Grading for hairiness was done in the field on one leaf per plant. The first fully expanded leaf was used at about the commencement of flowering. Because of the two-level hairiness it was necessary to grade for both length and density. Grades 0-8 were used for density and grades 1-8 for length. Four surfaces were examined, lower leaf lamina, upper leaf lamina, stem or petiole and upper main vein surfaces. Studies on hairiness in cotton have been in progress at Shambat for some time and it has been found necessary to include all these surfaces for an adequate analysis of the inheritance of hairiness.

Since the classes "Fully Hairy" and "Glabrescent+Slightly Hairy" are unmistakably distinct the data will not be presented under the grades recorded in the field but grouped under these heads.

The small F_2 families gave very little guidance as to the true nature of the segregation. However, the bulked data (26 Fully Hairy to 64 Glabrescent) suggest a single gene difference between dense and very sparse hair.

The F_3 families leave no doubt that this interpretation is justified. The entire F_2 family G233/56, segregating 11 glabrescents to 2 fully hairiness, was individually selfed and the results are presented in table 4.

Of the 11 glabrescents in G233/56, 3 were found to be homozygous glabrescent, 7 segregated for hair and one produced no seed, the 2 fully hairies breeding true. Thus G233/56 gave 3 homozygous glabrescents, 7 heterozygous hairiness and 2 fully hairies. This is in good agreement with an expectation of 3·0, 6·0 and 3·0 for the same classes for a 1 : 2 : 1 ratio. In most segregating families (see table 4)

TABLE 4

F_3 families from the cross WB1-1 × WB1-14

TABLE 4

F_3 families from the cross WB1-1 × WB1-14

Parent Genotype	G family 1957	Observed			Parent plants of families G/57					Description of two-level hairiness of families G/57	
						Hair grading * Density/Length (e.g. 6^6)					
		Glabrescent	Slightly hairy	Fully hairy	Parent Plant no. G233/56	L.L.L.	U.L.L.	S a.	S b.	U.M.V.	Short stellate	Long simple
H^{A1} H^{A1}	WB1-1			*	Average grade	6^1	6^1	3^2	6^6	3^2	Present	Dense
H^{A2} H^{A2}	WB1-14	*				1^1	2^1	3^2	o	1^2	Present	Absent
H^{A1} H^{A2}	F_1		*			2^1	2^2	3^2	3^5	1^2	Present	Present
H^{A1} H^{A1}	218			83	2	7^2	7^2	6^2	2^5	3^2	Dense	Sparse
	224			87	8	6^2	6^2	o	6^5	4^4	Moderate	Dense
	217	28	50	25	1	2^2	2^2	o	4^5	1^3	Absent or observed	Preponderant
	221	36	34	20	5	2^2	2^2	4^2	2^6	1^2	Preponderant	Occasional
	223	11	28	16	7	1^2	1^2	3^2	2^5	1^1	Segregating	
H^{A1} H^{A2}	225	28	64	34	9	2^2	2^2	o	3^5	1^2	Usually present	Preponderant
	226	21	43	21	11	1^2	1^2	o	3^4	1^2	Moderate but less dense than long	Preponderant
	227	20	60	26	12	3^2	3^2	2^2	4^5	1^2	Segregating	
	228	15	12	5	13	1^3	2^3	3^2	1^4	1^2	Preponderant	Moderate but less dense than short
H^{A2} H^{A2}	219	46			3	o	1^2	o	1^5	o	Absent	Occasional
	220	57			4	1^1	2^2	1^2	2^5	1^1	Occasional	Absent
	222	38			6	1^1	1^2	1^2	2^5	1^1	Glabrous	

* L.L.L. = Lower Leaf Lamina, U.L.L. = Upper Leaf Lamina, S. = Stem or Petiole ; a. = short stellate, b. = long simple, U.M.V. = Upper Main Vein.

it was possible to place plants into each of the three classes, but in two families, G221/57 and G228/57 glabrescent and slightly hairy were too close in phenotype for accuracy in separating the two classes. There was of course no doubt at any time concerning the fully hairy class. The figures require no comment since in all cases the agreement with expectation is excellent. Sparse hair and dense hair are therefore due to allelic genes and the following symbols are proposed, H^{A1} for the densely hairy condition of WB1-1 which is recessive to H^{A2}, the sparsely hairy condition of WB1-14. This scheme allows for the possibility of other alleles at the same locus being discovered. However, a revision may be necessary should it be possible to test the homology of this locus with those of the New World or Asiatic cottons.

TABLE 5

F_2 and F_3 families of the cross WB1-1 × WB1-14

		Bulk of families G233/56 to G241/56		G223/57		G227/57	
		Hairy	Glabrescent	Hairy	Glabrescent	Hairy	Glabrescent
Long simple preponderant .	Observed	7	23	11	7	6	25
Long simple/short stellate No preponderance . .	Observed	13	26	2	26	10	47
Short stellate preponderant .	Observed	6	14	6	6	9	9
Totals	Observed	*26*	*63*	*16*	*39*	*25*	*81*

WB1-1 and WB1-14 differed also in one other respect with regard to hair. WB1-1 possessed two levels of hairiness on the vascular tissues and WB1-14 only one. An examination of the grading for length and density of stem hair showed that this condition was under apparently simple control, *i.e.* a single factor determines the relative amounts of long simple hairs and short stellate ones on the stem and petiole. The data were divided into three classes where segregation for this factor was occurring. These classes were, 1. long simple hairs preponderant, stellate sometimes absent, 2. long simple and short stellate present in approximately equal densities and 3. short stellate preponderant, long sometimes absent. These classes corresponding to what might be expected to be found from the action of gene pairs *AA*, *Aa* or *aa* respectively on the H^A locus. All families showing variation for this character were plotted against segregation for hair density due to H^{A1} and H^{A2} (see table 5).

The bulked figures for the F_2 families give a very good agreement indeed with the expectation for independent segregation of two

factors except in the Long-equal/Glabrescent section. Here difficulties in grading glabrescents are thought to be contributory causes leading to error. Jointly long plus equal give a good 3 : 1 to short. G223/57 and G227/57 show large discrepancies at certain points in the tables but the individual 3 : 1 and 1 : 2 : 1 ratios are good. It is felt that in spite of the discrepancies there is very good evidence for the independent action of a gene affecting the two-level character of stem hair. The nature of the stem hair in those families not segregating for this character is noted in table 4, giving further evidence of its action. This modifier shows no dominance and its mode of action could be that of a suppressor controlling the production of a certain substance which determines the relative amounts of each hair type grown. The symbol proposed for this gene is therefore *Su*^{h}, i.e. hair suppressor, and represents the type found in WB1-1, namely, dominant long simple hairs, short stellate sparse or absent. It follows that *su*^{h}, represents dominant short stellate, long simple sparse or absent as in WB1-14.

4. DISCUSSION

Two new genes have been described for *G. anomalum* and a preliminary investigation into flower colour made. Apart from this variation the species is remarkably uniform. The extremely wide distribution of *G. anomalum*, stretching across the entire width of Northern Africa and occupying the S.W. of Southern Africa but discontinuous between these areas, might lead one to expect greater divergencies than are actually found. Since the natural habitat of the plant is extremely xerophytic each small group of wild plants is often separated from others by great distances. This is particularly true of the Sudan where as few as one or two plants may occupy a stony Jebel separated from the next Jebel by many miles of arid desert; thus the isolation of plants within the distribution is quite marked.

If a more intensive study were possible it is quite likely that plants collected from different localities, in particular those from the south in comparison with those from the north, would show differences in genetic balance due to selective response to locality. This is perhaps indicated in disturbances noticed in flower structure in the F_3 material from the cross between the northern WB1-1 and the southern WB1-14. The flowers of the parent strains, apart from colour, are identical, yet the hybrid material contained minutely petalled flowers, short corollas which did not open and from which the stigmas protruded, and lastly petalody.

Two other magenta-flowered accessions exist in the collection, both from the S.W. However, it is not known whether the flower colour mechanism within these is similar to WB1-1. No magenta-flowered *anomalum* is known in the north.

Wagner and Mitchell (1955) discuss very fully the types of modifiers reported in the literature. They say, " Many intermediate conditions exist which lie between the extreme examples of suppressor and complementary genes on the one hand, and genes which seemingly produce

their phenotypic effect completely independently of all other genes, on the other hand. Most of the intermediate types are best described by the general term modifiers. . . ." If the genotype acts as a co-ordinated whole, which presumably it must, then all genes are to some extent modifiers of one another—these effects are not readily observable except when the genotypic balance is disturbed by hybridization as is the case in the examples noticed here. The modification of the main gene type may have no significance beyond an indication of a disturbed genotypic balance which is ascribable to a unit gene or block of genes.

5. SUMMARY

1. Attention has been drawn to the uniformity of the species *G. anomalum* despite its extremely wide range and isolation within that range.

2. No final conclusions could be drawn about the genetics of flower colour. It was thought that at least one gene of large effect was present operating against a polygenic background which gives a wide range of colour types in hybrid populations.

3. A single gene difference between palmatifid and palmate leaf shape is described. Since the relationship with Silow's L^A has not been studied, no symbol is assigned to the new gene for palmately cut leaf. Evidence is presented for the existence of a gene affecting lobe width.

4. The hairiness of *G. anomalum* is basically due to control at a single locus. Dense hairiness is recessive to very sparse hairiness and the symbols H^{A1} and H^{A2} have been given to these conditions respectively. The two-level hairiness of the stem and petiole is clearly under monogenic control. This gene Su^h, has been so called because of its suppressor-type action.

5. The significance of the occurrence of modifiers was discussed.

Acknowledgment.—My thanks are due to Professor Sir Joseph Hutchinson for reading the manuscript and giving helpful criticism.

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NOTES AND COMMENTS

DIFFERENTIAL DAMAGE IN A MIXED COLONY OF THE LAND SNAILS *CEPAEA NEMORALIS* AND *C. HORTENSIS*

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The ecological relation between these two species has long been a puzzle. Most often they are found in separate colonies, occasionally in overlapping colonies, and rather rarely the colonies seem to occupy exactly the same area. Alkins (1922) noticed that overlapping colonies were commoner on limestone than off it. Oldham (1929) noted that joint colonies did not seem to occur on blown sand, and because of this Boycott (1934) allowed that the species might sometimes be in competition. Cartwright (1922); Dalgliesh (1930); Diver (1940); Lowe (1944) and Stratton (1950) all add information while confirming the pattern of colonies. Alkins' and Oldham's observations suggest that in poor habitats the species cannot live together, but as they both live separately in these habitats this is presumably because of competition. When they occur together is competition absent or merely reduced? This cannot be answered by simply observing the overlap of colonies; other evidence must be used. The facts I record below may help to answer the question.

In a small beech wood, the Shoulder of Mutton Plantation, on Hackpen Hill, Marlborough Downs (Nat. Grid Ref. SU 131742), both *nemoralis* and *hortensis* are found. On the east side near the track, but still in the wood, there is a nettle-bed and beyond this the floor of the wood is rather bare, cow-grazed and grassy. This part of the wood is on clay from the clay-with-flints beds. Both species occur in and outside the nettle-bed. On 12th April 1954 I visited the wood with Dr P. M. Sheppard. Both species were active and we collected all the live snails we could find in and around the nettles; most of them were crawling on the trees. Many of the shells were found to be damaged, some so badly that it was remarkable that the snails had lived to repair them, some so slightly that there was but a small crack, not of course to be confused with growth stoppage marks. It is uncertain how the shells became damaged; some of the cracks could have come from the snail falling off trees, others were undoubtedly the result of attacks by predators. Our total collection, classified by phenotype and damage, is given in table 1.

The numbers in most of the classes are rather small, so that most comparisons must be made with Fisher's exact test for 2×2 tables. The *hortensis* are predominantly yellow five-banded and the *nemoralis* pink unbanded so that the two species look very different, on the whole, in this sample. This is related to the background, the *nemoralis* being typical of that species in beech woods, while *hortensis* is often five-banded in this habitat (Cain and Sheppard, 1954). Although colonies with pink do occur in *hortensis*, they are uncommon and this could conceivably be related to the

occurrence of the various forms of *nemoralis*. The *nemoralis* are commoner outside the nettle-bed, the *hortensis* commoner inside it, and the probability that this is a chance distribution is much less than one in a thousand.

Much more surprising than these differences are those in the proportions of damaged shells in the two areas. In *hortensis* a greater proportion are

TABLE 1

Classification by phenotype and damage of Cepæa *collected from the Shoulder of Mutton Plantation. Y = yellow, P = pink, B = brown. 12345 = five-banded, 00300 = one-banded, 00 : 00 = with one interrupted band, 00000 = unbanded*

Nettle bed		Damaged	Undamaged	Total
C. hortensis	Y 00000	1	3	4
	Y 12345	6	23	29
	Total	*7*	*26*	*33*
C. nemoralis	Y 00000	1	1	2
	Y 00300	2	0	2
	P 00000	5	0	5
	Total	*8*	*1*	*9*
Grass area				
C. hortensis	Y 00000	1	0	1
	Y 12345	5	4	9
	Total	*6*	*4*	*10*
C. nemoralis	Y 00000	2	0	2
	Y 00300	2	1	3
	P 00000	8	7	15
	P 00300	1	4	5
	B 00000	0	1	1
	B 00 :00	0	1	1
	Total	*13*	*14*	*27*

damaged in the grassy part, in *nemoralis* a greater proportion in the nettles. These differences have probabilities of occurring by chance of 0·028 and 0·036 respectively. As in both cases snails are more often damaged in the area where their species is less common, the two probabilities may be combined to give a χ^2 (with four degrees of freedom) of 13·79 which is significant at the one per cent. level. This makes it reasonably certain that snails are more likely to become damaged in the area where the other species predominates. Until the causes of the cracks and breaks are known the import of this will be obscure, but at least it shows that even in mixed colonies there are differences between the species which may be an expression of competition.

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DURATION OF MEIOSIS IN RELATION TO TEMPERATURE

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1. INTRODUCTION

Knowledge of the time taken by meiosis is rather fragmentary and is sometimes given without specifying the temperature. The following examples of published data on the duration of meiosis illustrate this : *Lilium longiflorum* and *Gasteria*, 4 days (Marquardt, 1937 ; Straub, 1937) ; *Vicia faba*, 3 to 4 days (Marquardt, 1951) ; *Antirrhinum majus*, from the last premeiotic mitosis to tetrad, 30 to 34 hours (Ernst, 1938) ; *Tradescantia reflexa*, at room temperature, 18 to 23° C., 2 days (Sax and Edmonds, 1953) ; *T. paludosa*, at an unspecified room temperature, 52 hours (Steinitz, 1944) ; *Œnothera* at 10° C., 12 days, but from leptotene to metaphase I, only 6 days (Linnert, 1951). No attempt seems to have been made to determine the times taken by meiosis in the same organism over a wide range of temperatures. On the other hand, in mitosis Barber (1939) studied the rate of chromosome movement at anaphase in staminal hairs of *Tradescantia* at various temperatures, and much fuller data on plant and animal material have been provided by Hughes (1952). Brown (1951) determined the duration of various stages of cell division in root tips of *Pisum* at the temperatures of 15, 20, 25 and 30° C.

In the present paper an account is given of an attempt to determine the rate of complete meiosis (not the various stages) in pollen mother-cells of the Bluebell, *Endymion nonscriptus* (L.) Garcke at different temperatures, and to compare this with the rate of mitosis.

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2. EXPERIMENTAL PROCEDURE

The material came from Madingley Wood, Cambridge, during the winter of 1955-56. In *Endymion nonscriptus* the outermost anther, with respect to the floral axis, is commonly the most advanced in development but is generally closely followed in clockwise sequence (seen from above) by the other two outer anthers and the three inner ones in the same fashion (fig. 1). The differences between the age of the three outer anthers were small and were regarded for the present study as negligible. But there was a much more significant difference between the age of the youngest of the outer anthers and the oldest of the inner anthers. The latter were not used in the experiments.

After removing the upper half of the scales of a bulb to expose the inflorescence, the outermost anther in the lowest flower-bud was removed and its meiotic stage determined in an aceto-carmine smear. If this anther showed an early stage of meiosis, then the inflorescence (with the stem and roots of the bulb still attached to it) was labelled and placed on a pad of wet cotton-wool in a petri-dish and covered with a similar pad in the lid. Bulbs prepared in this way were kept in constant-temperature chambers at −5° C., −3° C., 0° C. and at intervals of 5° to 35° C. Others were kept at room temperature (15-21° C.). The plants were regularly watered through the cotton-wool, using water stored at the appropriate temperatures. At intervals the other two outer anthers in the bud were removed, examined as above, and the meiotic stage reached recorded.

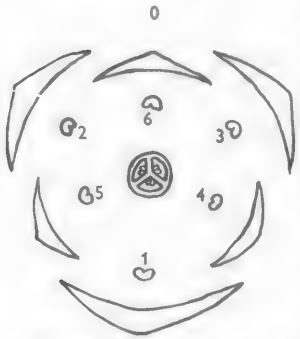

FIG. 1.—Floral diagram showing the usual (clockwise) order of progression of meiosis within the anthers of a flower-bud in the Bluebell. (Oldest and outermost anther is numbered 1; youngest and innermost anther, 6.)

3. EXPERIMENTAL RESULTS

No results were obtained at −5, −3 and +35° C., since at these temperatures the plants died before full meiosis was completed. The results for the remaining temperatures are set out in table 1. It is evident that the time taken from leptotene to metaphase I is about half the time taken for the whole of meiosis.

The duration of meiosis (Leptotene to Tetrad) at the various temperatures is as follows :

Temperature ° C.	0°	5°	10°	15°	20°	25°	30°
Time in Days (24 hrs.)	36·00	15·00	7·00	3·50	2·00	1·25	0·83

At room temperature (15-21° C.) meiosis took 2·75 days.

TABLE 1

Determinations of time taken by meiosis at different temperatures

Plant no.	Original stage	Time in days	Final stage
	0° C.		
1	Lept.	7	Pach.
2	Lept.	10	Pach.
3	Met. I	21	Tetrad
4	Lept.	20	Met. I
5	Lept.	24	Anaph. I
6	Lept.	20	Met. I
7	Lept.	22	Met.+ Anaph. I
8	Lept.	36	Tetrad
9	Lept.	36	Tetrad
	10° C.		
1	Lept.	5	Anaph. I
2	Lept.	4	Met. I
3	Lept.	6	Met. II
4	Diak.	5	Anaph. II
5	Lept.	3·5	Met. I
6	Lept.	7·0	Tetrad
7	Lept.	7·0	Tetrad
	20° C.		
1	Lept.	2	Met. II
2	Lept.	3	Tetrad
3	Lept.	1	Met. I
4	Diak.	1·5	Anaph. II
5	Diak.	1·5	Tet. I
6	Lept.	2·00	Tetrad
7	Lept.	2·00	Tetrad
	30° C.		
1	Lept.	36	(Destroyed)
2	Lept.	24	(Destroyed)
3	Lept.	20	Tetrad
4	Lept.	6	Dipl.
5	Lept.	12	Anaph. I
6	Lept.	20	Tetrad
7	Lept.	20	Tetrad

Plant no.	Original stage	Time in days	Final stage
	5° C.		
1	Lept.	10	Diak.
2	Lept.	10	Met. I
3	Lept.	11	Diak.
4	Lept.	12	Anaph. I
5	Lept.	6	Pach.
6	Dipl.	9	Tetrad
7	Lept.	16	Tetrad
8	Lept.	14	Tetrad
	15° C.		
1	Lept.	1·5	Met. I
2	Lept.	2	Tet. I
3	Met. I	2	Met. II
4	Lept.	1·5	Diak.
5	Met. I	2	Anaph. II
6	Lept.	3·5	Tetrad
7	Lept.	3·5	Tetrad
	25° C.		
1	Lept.	24	Tet. I
2	Lept.	26	Anaph. II
3	Anaph. I	10	Tetrad
4	Lept.	20	Anaph. I
5	Lept.	15	Met. I
6	Lept.	30	Tetrad
7	Lept.	30	Tetrad
	Room 15-21° C.		
1	Lept.	2	Anaph. I
2	Dipl.	2	Tet. I
3	Pach.	1	Met. I
4	Diak.	2	Anaph. II
5	Dipl.	2	Anaph. II
6	Dipl.	1·75	Met. II
7	Met.	1·75	Anaph. II
8	Lept.	2·75	Tetrad
9	Lept.	2·75	Tetrad

4. DISCUSSION

The data obtained on the duration of meiosis in *Endymion nonscriptus* at various temperatures agree broadly with the published results given above. Similar studies with other material would be interesting for comparison.

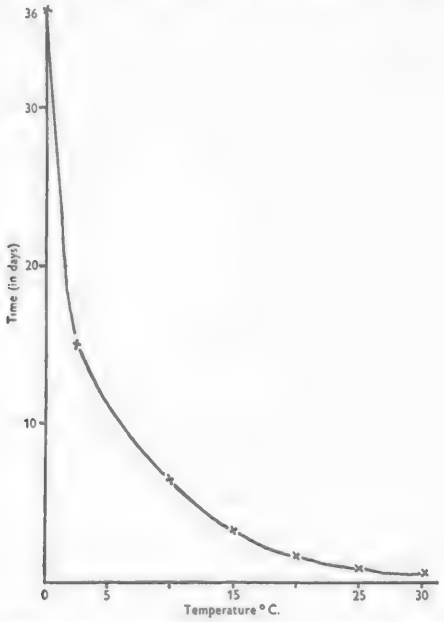

FIG. 2.—Graph showing the effect of temperature in accelerating the time taken by meiosis in the Bluebell.

A great acceleration in the rate of meiosis with increasing temperature is evident (fig. 2). Q_{10} values measured at 5° intervals (see Barber (1939) for method of calculation) are given below :

Temperatures ° C.	Q_5	log	X2	Q_{10}
0-5°	2·4000	0·3802	0·7604	5·759
5-10°	2·1428	0·3308	0·6616	4·588
10-15°	2·0000	0·3010	0·6020	3·999
15-20°	1·7500	0·2430	0·4860	3·062
20-25°	1·6000	0·2041	0·4082	2·560
25-30°	1·5060	0·1761	0·3522	2·250

These results are comparable to those for respiration in plant tissues, where Q_{10} values of 2·0 to 2·5 are usual within the range of 10° C. to 30° C., and with higher values at temperatures below 10° C. (Meyer and Anderson, 1952). Brown (1951) found that the mean duration of mitosis in *Pisum* at various temperatures was as follows : 15° C., 2·95 hours ;

20°, 1·83 hours ; 25°, 1·38 hours ; and 30° C., 1·09 hours. Thus, meiosis in *Endymion* anthers takes about 20 times as long as mitosis in *Pisum* at the same temperature. The Q_{10} values for the rate of mitosis from Brown's data, calculated in the same way as above, are : 15-20°, 2·598 ; 20-25°, 1·758 ; and 25-30°, 1·606. It is thus clear that, while in both meiosis and mitosis Q_{10} values fall with increasing temperature, the values are relatively lower in mitosis.

5. SUMMARY

1. The duration of meiosis in the pollen mother-cells of the Bluebell, *Endymion nonscriptus* (L.) Garcke, has been estimated for various temperatures ranging from 0° C. to 30° C.

2. Meiosis showed a rapid shortening of duration with increasing temperature, but the Q_{10} values fall as the temperature rises.

3. Meiosis in the Bluebell lasted about 20 times as long as mitosis in *Pisum* at the same temperature (data of Brown (1951)).

Acknowledgments.—The author is indebted to Dr H. L. K. Whitehouse and the authorities of the Botany School and the Low Temperature Research Station, Cambridge, for their help in advice and facilities.

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REVIEWS

GENETIC RESISTANCE TO DISEASE IN DOMESTIC ANIMALS. By F. B. Hutt. London: Constable. 1958. Pp. xii + 198. 35s.

Most textbooks on animal breeding, as Professor Hutt points out, make little or nothing of the possibilities of breeding for disease resistance. Now that he has marshalled much of the available evidence in a convenient and highly readable form, the significance of the omission can be assessed. He refers briefly to a wide range of subjects including lethal genes, adaptation to environment, aberrant metabolism, and disease resistance in large and small animals. At its worst the evidence is anecdotal; at its best, convincing. Some of it is open to conflicting interpretations.

Professor Hutt's argument for more attention to breeding for disease resistance rests heavily on his own and his colleagues' work with poultry in which, aided by a high reproductive rate, they have successfully demonstrated the practical value of genetic selection. For all his enthusiasm, however, he does not wish to pretend that, for livestock other than poultry, the factual basis for action is good. Observations and experiments have been mostly inadequate and sporadic. The reputation of the Romney Marsh sheep for resisting stomach worms, for example, appears to be supported only by five lambs in California. Nevertheless, ample reasons are found for supposing that genetic variation in susceptibility to disease occurs in all classes of livestock. The question is whether attempts should be made to exploit it. Conspicuous genetical errors of metabolism and development can be left to natural selection to oppose, aided, if need be, by culling relatives of the abnormal; but Professor Hutt thinks this will not do for the whole of the long catalogue of diseases against which veterinarians contend with vaccines, antibiotics, hygiene, undiscriminating slaughter and faith. His brave shouting in the poultry house, however, dwindles to whistling in the darkness of the sheepfold. If scrapie and mastitis have to be adduced as examples of diseases that might be controlled by breeding methods appropriate to leucosis in fowls, the present weakness of the case is evident.

A good deal more work on most diseases is needed before a competent choice for a long-term policy for any of them can be made from the techniques of prevention, slaughter, treatment and breeding.

The book is attractively produced, and an excellent investment.

H. P. Donald.

PLANT BREEDING AND CYTOGENETICS. By F. C. Elliott. McGraw-Hill Publishing Co. Ltd. 1958. Pp. 1–395. 66s.

In this volume the author has attempted to outline and describe the nuclear and reproductive mechanisms which control variability and the way in which they have been used to improve crop plants. Firstly, genetic mechanisms covering chromosome mechanics, recombination and breeding systems are described. Two chapters deal with the techniques involved in

the manipulation of progenies following crossing and selection. The problem of breeding for disease resistance is treated briefly in a separate chapter. The last part of the book is concerned with the testing and release of new selections and with the organisation of plant improvement programmes. This latter section is unrelated to the rest of the text and it is difficult to see why it was included in the present work.

The author deals with an impressively wide field, and presents a bewildering array of facts and quotations which are fully documented. Some of the material, in particular the achievements derived from the use of polyploids, artificial mutations and chromosome substitution, appears in book form for the first time and the coverage given to it will be generally appreciated. In attempting a complete survey of the literature the author has, however, produced a form of review in which the fundamental principles have been buried within semi-digested quotations and references. Where a fully illustrated explanation of basic laws is expected, one finds the treatment given here to be very thin and superficial. In the section on incompatibility, for example, the sporophytic mechanism merits only a paragraph and the subject is dismissed with the statement: "A great complexity of incompatibility relationships is possible because of the variability of allelic interactions" (page 30). Apomictic mechanisms are treated in the same summary fashion and the reader is referred to other authors for examples, for details, as well as for the historical development of the subject.

On countless occasions the reader is led to the very edge of the subject-matter only to find that what is really important is not here at all but elsewhere. For differences in recombination between central and marginal populations one is invited to see Carson, 1955; for the several doubled flowers already in commercial trade channels one must consult Emsweller, 1948, and for the important evolutionary consequences that accrue from changes in population size under the various environments encountered by a species, one has to read Ford, 1955. Similar instances occur with such regularity that the text frequently becomes no more than an index to the literature. Had the facts been more thoroughly digested and the principles of the subject separated from the opinions of various authors, this unfortunate presentation could have been avoided and the work might have attained the level of a textbook. WATKIN WILLIAMS.

VARIABLES RELATED TO HUMAN BREAST CANCER. By V. E. Anderson *et al.* London. Minnesota University Press, Oxford University Press. 1958. Pp. xiii + 172. 32s.

An account of a study of familial incidence of breast cancer in Minnesota. No one-egg twins were included in the study but, instead, a table is given to show what results would have been obtained from one-egg twins if they had been studied. This table is based on assumptions that such twins would show complete concordance in presence or absence of breast cancer but no more concordance than random pairs in the general population of patients in the time of onset. The authors seem to have overlooked the available evidence which bears on these assumptions, and indeed invalidates them.

Altogether this study is not well conceived and it is not surprising that, after prolonged exertions, the authors fail to reach any serious conclusion.

C. D. D.

A GUIDE TO FIELD BIOLOGY. By John Sankey. London: Longmans. 1958. Pp. xvii + 166. 12s. 6d.

This is a lucid introduction to the field study of plants and animals for use in schools. The bibliography is a helpful one within the limits imposed by the text. The style is straightforward and clear, and the book is obviously written by someone with practical experience.

It is astonishing, however, that an ecological textbook to-day, however simple, should omit genetics, which this does completely. So much attention is given to the differences between species that it is a pity to omit the differences within them. For these could easily be studied by beginners and are useful in training their powers of observation and inference.

The principles of genetics can easily be explained so as to be grasped at an early age; there are also introductory works for the purpose to which reference could have been made. Thus equipped, children should be shown as this book should show them, that there exist situations in which evolution occurs so quickly that its results can convincingly be demonstrated in the English countryside. Darwin, who is mentioned in the foreword, could then be referred to in the text. It is disappointing to see, in a book such as this, a list of Projects which omits studies on genetics and on natural selection. Very suitable examples could be found: the selective elimination by birds of colour and banding patterns in snails; work on heterostyle plants and other examples of polymorphism, with an elementary account of the significance of that condition; observations on polygenic variation of the Meadow Brown butterfly and (useful in many parts of the country) upon industrial melanism. If these particular instances had not found a place here, other comparable ones should have done so.

The technique of marking, release and recapture is not well handled. The explanation is too condensed, and should be illustrated by an example. Also, simplification should not involve inaccuracy. It is not correct to say (page 106) that "this method" (by which anyone would understand marking, release and recapture) "does not allow for fluctuations in the population due to death and migration or emergence of fresh adults". The elementary calculation here shown certainly throws no light on such population changes, but children should at least be told that information upon them can be deduced from such work by a more refined analysis.

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